

EP 29264 7

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/34614 A2

(51) International Patent Classification⁷: C07F 9/02 Susan, L. [US/US]; 1225 Martha's Chapel Road, Rt. 3, Box 184P, Apex, NC 27502 (US).

(21) International Application Number: PCT/US00/41352

(22) International Filing Date: 20 October 2000 (20.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 60/162,290 28 October 1999 (28.10.1999) US

(71) Applicants (for all designated States except US): WAKE FOREST UNIVERSITY [US/US]; Medical Center Boulevard, Winston-Salem, NC 27157-1023 (US). THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL [US/US]; Office of Technology Development, 308 Bynum Hall, Campus Box 4105, Chapel Hill, NC 27599-4105 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KUCERA, Louis, S. [US/US]; 4860 Ellen Avenue, Pfafftown, NC 27040 (US). FLEMING, Ronald, A. [US/US]; 1005 Greenly Drive, Pfafftown, NC 27040 (US). ISHAQ, Khalid, S. [US/US]; 105 Hunter Hill Place, Chapel Hill, NC 27514 (US). KUCERA, Gregory, L. [US/US]; 705 Manly Street, Winston-Salem, NC 27101 (US). MORRIS-NATSCHKE,

(74) Agents: DOYLE, Kathryn et al.; Akin, Gump, Strauss, Hauer & Feld, L.L.P., Suite 2200, One Commerce Square, 2005 Market Street, Philadelphia, PA 19103-7086 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— Without international search report and to be republished upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPOSITIONS AND METHODS FOR DOUBLE-TARGETING VIRUS INFECTIONS AND TARGETING CANCER CELLS

WO 01/34614 A2

(57) Abstract: The invention includes compositions and methods useful for treatment of a virus infection in a mammal by double-targeting the virus (i.e. targeting the virus at more than one stage of the virus life cycle) and thereby inhibiting virus replication. The compositions of the invention include compounds which comprise a phosphocholine moiety covalently conjugated with one or more antiviral agents (e.g. nucleoside analogue, protease inhibitor, etc.) to a lipid backbone. The invention also includes pharmaceutical compositions and kits for use in treatment of a virus infection in mammals. The methods of the invention comprise administering a compound of the invention, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the invention, in an amount effective to treat the infection, to a mammal infected with a virus. Additionally, the invention includes compositions and methods useful for combating a cancer in a mammal and for facilitating delivery of a therapeutic agent to a mammalian cell. The compositions of the invention include compounds which comprise an alkyl lipid or phospholipid moiety covalently conjugated with an anticancer agent (e.g. a nucleoside analogue). The invention also includes pharmaceutical compositions and kits for combating a cancer and for facilitating delivery of a therapeutic agent to a mammalian cell. The methods of the invention comprise administering a compound of the invention, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the invention, in an amount effective to combat a cancer or to facilitate delivery of a therapeutic agent to a mammalian cell.

TITLE OF THE INVENTION

COMPOSITIONS AND METHODS FOR DOUBLE-TARGETING VIRUS
INFECTIONS AND TARGETING CANCER CELLS

BACKGROUND OF THE INVENTION

Acquired immunodeficiency syndrome (AIDS) is a degenerative disease of the immune system and central nervous system (CNS) resulting from infection of humans by HIV virus. AIDS is responsible for a rapidly growing fatality rate in the world population. At present, no cure has been found, and clinically approved drugs are limited in number. These drugs include nucleoside reverse transcriptase (RT) inhibitors such as 3'-azido-3'-deoxythymidine (AZT, Zidovudine), dideoxyinosine (ddI, Didanosine), dideoxycytidine (ddC, Zalcitabine), 2', 3'-dideoxy-3'-thiacytidine (3TC, Lamivudine), and 2', 3'-didehydro-3'-deoxythymidine (d4T, Stavudine), a non-nucleoside RT inhibitor (Niverapine), and protease inhibitors such as saquinavir (Inverase), ritonavir (Norvir), indinavir (Crixivan), and nelfinavir (Viracept). Nucleoside RT inhibitors generally have similar structures (2', 3'-dideoxynucleosides) and act at an early stage in virus replication to inhibit provirus DNA synthesis (De Clercq, 1995, *Journal of Medicinal Chemistry*, 38:2491-2517). However, AZT, the recommended initial therapeutic agent, and the other nucleoside analogues have several limitations, including adverse side effects such as bone marrow depression and anemia (Gill et al., 1987, *Annals of Internal Medicine*, 107:502-505; Richman et al., 1987, *New England Journal of Medicine*, 317:192-197). Peripheral neuropathy is also a major and common side effect. AZT is rapidly eliminated from the plasma with a half-life of about one hour (Surbone

et al., 1988, *Annals of Internal Medicine*, 108:534-540) and is quickly metabolized in the liver to its corresponding 5'-glucuronide, which is inactive.

Presently, only a small number of antiviral drugs are available for treatment of virus infections. A complication to the development of such drugs is that mutant strains of virus which are resistant to currently available antiviral drugs are developing at an alarming rate. Combinations of new drugs having unique modes of action are urgently needed to replace drugs that have lost their potency against viruses as a result of virus mutations. A further complication to the development of antiviral drugs is that development of viral resistance to available compounds is not the same in different body compartments and fluids. For example, evolution of drug resistance among HIV-1 clinical isolates is often discordant in blood and semen of HIV-1 positive males (Eron et al., 1998, *AIDS* 12:F181-F189).

Further, currently available drugs useful for antiviral therapy sometimes ineffectively penetrate the genital tract. This is a serious drawback to the use of these drugs to combat viruses which infect the genital tract. If an antiviral drug promotes development of resistance in the genital tract and the virus is commonly transmitted from this body site, the drug will rapidly become ineffective for treatment of the virus infection in the population at risk for transmission. Hence, drug-resistant mutants of certain viruses can be rapidly spread by sexual contact in the human population. It is known that viruses such as HIV, hepatitis B, hepatitis C, herpes simplex virus, cytomegalovirus, papilloma viruses, and many others are transmitted via sexual contact by both males and females. Thus, therapeutic drugs that fully suppress virus infections in the genital tract are a high public health priority.

Another limitation of presently available antiviral drugs is that rapid emergence of drug resistant mutant virus can lead to decreased sensitivity to the drug within a patient or within a patient population (Larder et al., 1989, *Science*, 243:1731-1734). Thus, the beneficial effects of drugs such as AZT are limited in duration.

The anti-HIV chemotherapy era which started a decade ago has recently made significant progress toward better control of HIV-1 infection by the introduction of protease inhibitors and the use of combinations of nucleoside and non-nucleoside RT inhibitors with protease inhibitors. Monotherapy (e.g. administration of a single drug) using a nucleoside or non-nucleoside RT inhibitor or a protease inhibitor is no longer a recommended form of **therapy for treatment of a patient with a virus infection such as HIV-1 infection**. Although combinations of AZT, 3TC, and a protease inhibitor have reduced virus load in the plasma of

patients to below detectable levels (i.e. fewer than 200 copies of viral RNA per milliliter of plasma) with a concomitant increase in CD4⁺ cell count, some drug combinations have been associated with increased toxicity in a person receiving multiple drug therapies. Also, although reduction in virus burden in the plasma of patients to non-detectable levels achieved using some 5 drug combinations is impressive, drug resistance is an escalating problem due to both use and misuse of drug therapy (De Clercq, 1995, Journal of Medicinal Chemistry, 38:2491-2517; Bartlett, 1996, Infectious Diseases in Clinical Practice, 5:172-179) and evolution of resistant mutants in blood and seminal fluids (Eron et al., 1998, AIDS, 12:F181-F189).

The pathogenic events in HIV disease have recently been reviewed by Fauci 10 (1996, Nature {New Biology}, 384:529-534). The current understanding is that entry of HIV into cells varies with the virus strain and cell type. Primary infection of humans is associated with macrophage tropic (M-tropic) virus that utilize the CD4 receptor and a beta-chemokine co-receptor (CCR5) for entry into macrophages. As HIV infection progresses, the initial M-tropic viruses are usually replaced by T-tropic viruses that enter T-lymphocytes via the CD4 receptor 15 and co-receptor CXCR4 (fusin). The viral determinant of cellular tropism maps to the gp 120 subunit of HIV-1 Env protein, particularly the 3rd variable region or V3 loop of gp120. Upon entry into these cells, HIV probably infects dendritic cells, which then carry the virus to CD4⁺ cells in the lymphoid organs. Infection is then established in the lymphoid organs and a burst of infectious virus seeds itself throughout the body, including the CNS, brain, and lymphoid 20 tissues and sexual organs (e.g. testes). Current drugs used in therapies for HIV infection and AIDS noted above have a limited capacity and half-life for absorption from the stomach to the blood, accumulation into lymphoid organs, crossing the blood-brain barrier into the CNS, or entering the sexual organs (e.g. testes) to attack sanctuaries for HIV replication.

Synthetic phosphocholine lipid (PC lipid) analogues such as, for example, 1-decanamido-2-decyloxypropyl-3-phosphocholine (INK-11) have demonstrated a low incidence 25 of unwanted side effects in mice such as reduction of bone marrow precursor cells and have exhibited high differential selectivity (i.e. the ratio of TC₅₀ for cytotoxicity to EC₅₀ for antiviral activity, DS=1342 for INK-11) in human leukocytes in cultured cells. At a dosage of 50 milligrams per kilogram of body weight per day for 21 days, INK-11 inhibited Friend leukemia 30 virus-(FLV-) induced pathogenesis by 42% in infected mice, as indicated by significant activity against splenomegaly. The observation that use of INK-11 resulted in only moderate suppression against RT activity compared with AZT alone (42% vs 98%, respectively) suggests

that INK-11 induces production of defective virus, similar to the effect achieved using other lipid compounds alone (Kucera, et al., 1990, AIDS Research & Human Retroviruses 6:491-501).

Other synthetic phospholipids which do not comprise a phosphocholine moiety

5 (non-PC lipids) have been conjugated with antiviral chemotherapeutic agents. For example, thioether lipid-nucleoside conjugates have exhibited improved antineoplastic activity in tumor-bearing mice (Hong et al., 1990, Journal of Medicinal Chemistry 33:1380-1386). Also, natural phospholipids coupled to AZT or to dideoxynucleosides (ddT, ddC) have proven to be markedly active against HIV by inhibiting viral RT activity (Steim et al., 1990, Biochemical &

10 Biophysical Research Communications 171:451-457; Hostetler et al., 1990, Journal of Biological Chemistry 265:6112-6117; Hostetler et al., 1991, Journal of Biological Chemistry 266:11714-11717). Studies of phospholipid antiviral efficacy have also included chemically conjugating AZT or ddI, through a phosphate-ester bond, to selected synthetic phosphatidic acid lipid analogues (Piantadosi et al., 1991, Journal of Medicinal Chemistry 34:1408-1414).

15 Synthetic phosphate-ester linked lipid-nucleoside conjugates were found to be markedly active against infectious HIV-1 production in both acutely- and persistently- infected cells, and were 5- to 10-fold less cytotoxic compared with AZT alone (Piantadosi et al., 1991, Journal of Medicinal Chemistry 34:1408-1414). Results of preliminary studies indicated that synthetic lipid-AZT conjugates block reactivity of HIV-1-induced gp160/gp120 proteins with specific

20 monoclonal antibodies on the surface of infected and treated cells and on the surface of treated HIV-1 particles, as measured by flow cytometry. These conjugate compounds also caused inhibition of HIV-1-induced cell fusion (Kucera et al., 1992, In: Novel Membrane Interactive Ether Lipids With Anti-Human Immunodeficiency Virus Activity, Aloia et al., eds., Membrane Interactions of HIV, pp.329-350; Krugner-Higby et al., 1995, AIDS Research & Human

25 Retroviruses 11:705-712). However, these phosphate ester-linked lipid-AZT conjugates (non-PC lipid-AZT conjugates) were not very active against AZT-resistant clinical isolates of HIV-1. Moreover, after intracellular metabolism of the conjugate with resulting release of AZT-monophosphate, the lipid moiety exhibited only moderate to non-detectable antiviral activity (Piantadosi et al., 1991, Journal of Medicinal Chemistry 34:1408-1414).

30 As with the antiviral agents, the development of anticancer agents for treating **cancer effectively has also been problematic. Barriers such as cellular mechanisms of** anticancer drug resistance, overcoming the blood-brain barrier to provide adequate delivery of

drug to the brain and CNS, inadequate uptake of drug by lymphoid and hematopoietic tissues, toxicity, achieving oral bioavailability, overcoming short drug half-life, and preventing extracellular metabolism of the anticancer agent are faced by the skilled artisan.

In order to improve bioavailability to CNS and brain tissue, nucleoside 5 analogues have been encapsulated in liposomes or used with modifying agents to disrupt the blood-brain barrier (Braekman, et al., 1997, Proc. Amer. Soc. for Clinical Oncology, Abstract #810). Implantable devices have been used to provide more sustained drug delivery to increase the pharmacokinetics of anticancer agents (Del Pan, et al., 1997, Proc. Amer. Soc. for Clinical Oncology, Abstract #1384). Additionally, attempts to improve the efficacy of nucleoside 10 analogues in cancer therapy have included the use of multidrug combinations and high-dose nucleoside analogue therapy (Capizzi, 1996, Investigational New Drugs 14:249-256). None of these methods have adequately overcome the problems discussed above with regard to anticancer agents.

Another attempt to circumvent the problems associated with conventional 15 nucleoside analogue cancer therapy has been the conjugation of these molecules to phospholipids. Thus far, the conjugation of nucleoside analogues to phospholipid molecules has focused on ara-C and a limited number of diacyl, alkylacyl and thioether phospholipids (Hong, 1990, Cancer Res. 50:4401-4406). Although these conjugates have shown efficacy in the treatment of hematologic malignancies, these drugs must be administered intraperitoneally 20 or intravenously and do not overcome the problems discussed above regarding anticancer agents. These conjugates are degraded by phospholipase A and phospholipase B extracellularly and do not provide the option of oral administration.

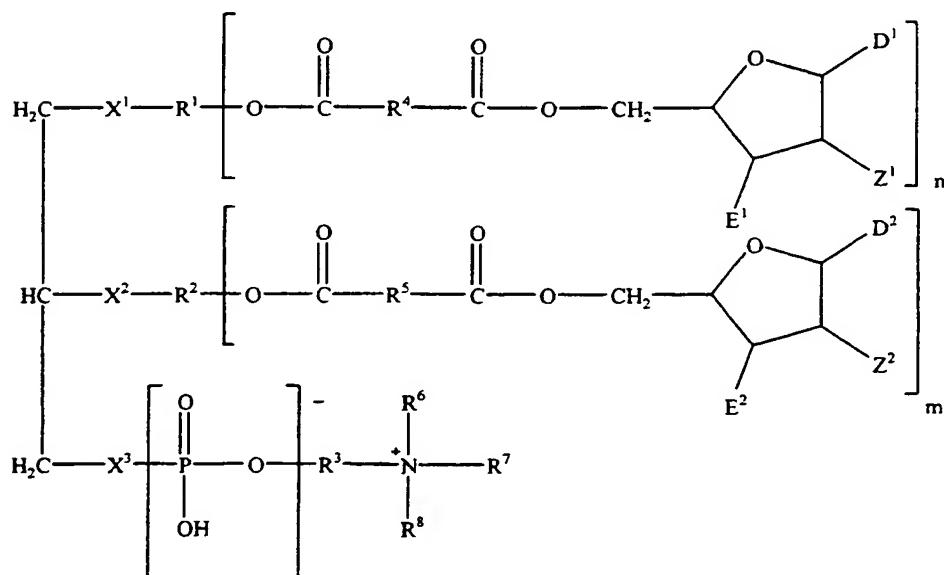
Despite the promising attributes of compounds such as PC lipids, and non-PC 25 lipid-nucleoside analogue conjugates, currently available antiviral and anticancer agents such as nucleoside analogues and anti-HIV nucleoside drugs have severe inherent limitations. Although such drugs are capable of delaying the onset of symptoms of virus infection and extending survival time for patients, new compounds having the attributes of increased tolerability, potency, and selectivity against specific viruses, differential mechanisms of action, ability to cross the blood-brain barrier, and freedom from myelosuppressive side effects are 30 urgently needed for improved treatment of virus infections. Also, new antiviral and anticancer compounds are needed which more effectively combat cancers or target multiple aspects of the virus life cycle, which facilitate delivery of an anticancer agent to cells and tissues not normally

accessible to anticancer agents (e.g. CNS and lymphoid tissues), which combine lipophilic (e.g. phospholipid) and antiretroviral or anticancer agents within the same molecule (e.g. conjugate compounds) in order to yield a drug with a more sustained antiviral or anticancer effect, which decrease the rate of emergence of drug-resistant virus strains, and which inhibit virus

5 replication in a wider range of cellular or tissue reservoirs of virus infection. The present invention satisfies these needs.

BRIEF SUMMARY OF THE INVENTION

The invention includes a compound having the structure of Formula I:



10 wherein,

n and m are each independently 0 or 1, but n and m are not both 0;

R¹ is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl if n is 0 and (C₁-C₁₆) alkylene, branched alkyl, alkenyl or alkynyl if n is 1;

15 R² is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl if m is 0 and (C₁-C₁₆) alkylene, branched alkyl, alkenyl or alkynyl if m is 1;

R³, R⁴ and R⁵ are each independently (C₁-C₈) alkylene;

R⁶, R⁷ and R⁸ are each independently (C₁-C₈) alkyl;

X¹ and X² are each independently S, O, NHC=O, OC=O or NH;

X³ is O or S;

20 E¹ is H, S, halo or N₃;

Z^1 is H, S, or halo; or E^1 and Z^1 together are a covalent bond;
 E^2 is H, S, halo, or N_3 ;
 Z^2 is H, S, or halo; or E^2 and Z^2 together are a covalent bond;
 D^1 and D^2 are each independently selected from the group consisting of purine,
5 pyrimidine, adenine, thymine, cytosine, guanine, hypoxanthine, inosine, uracil and ring
modifications thereof, including O, N, and S substitutions, and
wherein, each alkyl, alkylene, branched alkyl, alkenyl, alkynyl, adenine,
thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^1 , R^2 , R^3 ,
 R^4 , R^5 , R^6 , R^7 , R^8 , D^1 , and D^2 can, optionally, be substituted with 1, 2, 3, or 4 substituents
10 independently selected from the group consisting of halo, nitro, trifluoromethyl, (C_1 - C_8) alkyl,
(C_1 - C_8) alkoxy, aryl, and $N(R^a)(R^b)$ wherein R^a and R^b are each independently selected from
the group consisting of H and (C_1 - C_8) alkyl.

In one aspect, the compound is present in an amount effective to inhibit virus
replication in a mammal.

15 In another aspect, R^1 is (C_6 - C_{16}) alkyl if n is 0 or
-CH=CH- if n is 1.

In yet another aspect, R^2 is (C_6 - C_{16}) alkyl if m is 0 or
-CH=CH- if m is 1.

In a further aspect, R^3 is -CH₂CH₂-.

20 In one embodiment, R^4 is -CH₂-.

In another embodiment, R^5 is -CH₂-.

In yet another embodiment, R^6 , R^7 and R^8 are each -CH₃.

In one aspect, X^1 is S, NHC=O, -NH- or O.

In another aspect, X^2 is S, NHC=O or O.

25 In a further aspect, X^3 is O or S.

In another aspect, E^1 is N_3 , S or H.

In one embodiment, Z^1 is H or S.

In another embodiment, E^2 is N_3 , S or H.

In a further embodiment, Z^2 is H or S.

30 In one aspect, n is 0 and m is 1.

In another aspect, n is 1 and m is 0.

In yet another aspect, D¹ is selected from the group consisting of cytosine, guanine, inosine and thymine.

In a further aspect, D² is selected from the group consisting of cytosine, guanine, inosine and thymine.

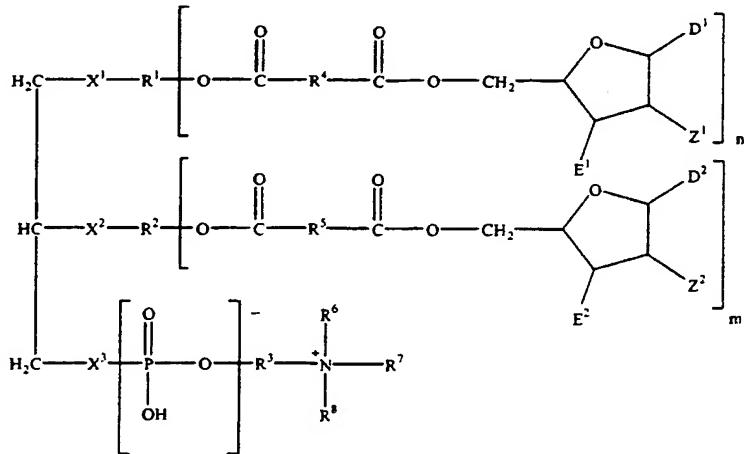
5 In another aspect, the compound is in the form of a pharmaceutically acceptable salt.

In one embodiment, the compound is present in an amount effective to inhibit virus replication in a mammal.

In a preferred embodiment, R¹ is (C₆-C₁₆) alkyl, branched alkyl, alkenyl or 10 alkynyl; R² is (C₄-C₁₂) alkylene; R³ is -CH₂CH₂-; R⁵ is -CH₂-; R⁶, R⁷ and R⁸ are each CH₃; X¹ and X² are each independently S, O or NHC=O; E² is H or N₃; D² is selected from the group consisting of thymine, cytosine, guanine and inosine, and wherein each alkyl, branched alkyl, alkylene, alkenyl, alkynyl, thymine, cytosine, guanine, and inosine of R¹, R², R³, R⁵, R⁶, R⁷, R⁸, and D² can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from 15 the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b), wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

The invention also includes a method of treating a virus infection in a mammal.

20 The method comprises administering to the mammal, in an amount effective to treat the infection, a compound having the structure of Formula I:



wherein,

n and m are each independently 0 or 1, but n and m are not both 0;

R^1 is (C_1 - C_{16}) alkyl, branched alkyl, alkenyl or alkynyl if n is 0 and (C_1 - C_{16}) alkylene, branched alkyl, alkenyl or alkynyl if n is 1;

R^2 is (C_1 - C_{16}) alkyl, branched alkyl, alkenyl or alkynyl if m is 0 and (C_1 - C_{16}) alkylene, branched alkyl, alkenyl or alkynyl if m is 1;

5 R^3 , R^4 and R^5 are each independently (C_1 - C_8) alkylene;

R^6 , R^7 and R^8 are each independently (C_1 - C_8) alkyl;

X^1 and X^2 are each independently S, O, $NHC=O$, $OC=O$ or NH;

X^3 is O or S;

E^1 is H, S, halo or N_3 ;

10 Z^1 is H, S, or halo; or E^1 and Z^1 together are a covalent bond;

E^2 is H, S, halo, or N_3 ;

Z^2 is H, S, or halo; or E^2 and Z^2 together are a covalent bond;

15 D^1 and D^2 are each independently selected from the group consisting of purine, pyrimidine, adenine, thymine, cytosine, guanine, hypoxanthine, inosine, uracil and ring modifications thereof, including O, N, and S substitutions, and

15 wherein, each alkyl, alkylene, branched alkyl, alkenyl, alkynyl, adenine, thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , D^1 , and D^2 can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C_1 - C_8) alkyl, (C_1 - C_8) alkoxy, aryl, and $N(R^a)(R^b)$ wherein R^a and R^b are each independently selected from the group consisting of H and (C_1 - C_8) alkyl.

20 In one aspect, R^1 is (C_8 - C_{12}) alkyl if n is 0 and
- CH_2CH_2 - if n is 1.

25 In another aspect, R^2 is (C_8 - C_{12}) alkyl if m is 0 and
- CH_2CH_2 - if m is 1.

30 In yet another aspect, R^3 is - CH_2CH_2 -.

In one embodiment, R^4 is - CH_2 -.

In another embodiment, R^5 is - CH_2 -.

In a further embodiment, R^6 , R^7 and R^8 are each - CH_3 .

30 In another embodiment, X^1 is S or O.

In one aspect, X^2 is S or O.

In another aspect, X^3 is O.

In a further aspect, E¹ is N₃ or H.

In a still further aspect, Z¹ is H.

In one embodiment, E² is N₃ or H.

In another embodiment, Z² is H.

5 In yet another embodiment, n is 0 and m is 1.

In one aspect, n is 1 and m is 0.

In another aspect, D¹ is selected from the group consisting of cytosine, guanine, inosine, and thymine.

10 In a further aspect, D² is selected from the group consisting of cytosine, guanine, inosine, and thymine.

In a still further aspect, the virus infection is an infection by a virus selected from the group consisting of HIV, hepatitis virus, and a herpes virus.

In one embodiment, the HIV is selected from the group consisting of HIV-1 and HIV-2.

15 Preferably, the hepatitis virus is selected from the group consisting of hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E viruses.

Preferably, the herpes virus is selected from the group consisting of herpes simplex virus type 1, herpes simplex virus type 2, varicella-zoster virus, cytomegalovirus, Epstein Barr virus, human herpes virus type 6, human herpes virus type 7, and human herpes 20 virus type 8.

In a preferred aspect, R¹ is (C₆-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl; R² is (C₄-C₁₂) alkylene; R³ is -CH₂CH₂-; R⁵ is -CH₂-; R⁶, R⁷ and R⁸ are each CH₃; X¹ and X² are each independently S, O or NHC=O; E² is H or N₃; D² is selected from the group consisting of thymine, cytosine, guanine and inosine, and wherein each alkyl, branched alkyl, alkylene, 25 alkenyl, alkynyl, thymine, cytosine, guanine, and inosine of R¹, R², R³, R⁵, R⁶, R⁷, R⁸, and D² can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b), wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

30 In one aspect, a pharmaceutically acceptable salt of the compound is administered to the mammal.

Preferably, the mammal is a human.

The invention also includes a method of inhibiting virus replication in a cell. The method comprises administering to the cell a compound of Formula I in an amount effective to inhibit virus replication in the cell.

5 The invention includes a pharmaceutical composition comprising a compound of Formula I in combination with a pharmaceutically acceptable carrier.

In one aspect, the compound is present in an amount effective to inhibit virus replication in a mammal.

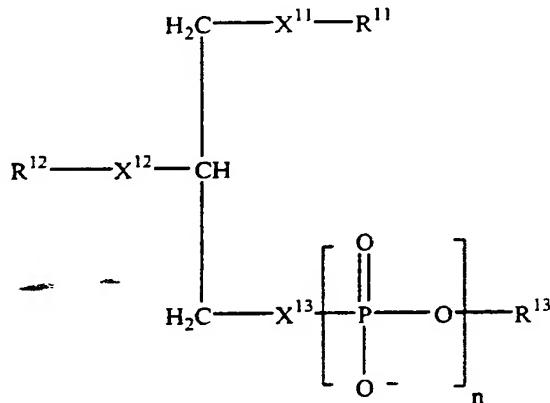
In another aspect, the compound is present in an amount effective to inhibit virus replication in a mammal.

10 The invention also includes a kit for treatment of a viral infection in a mammal.

The kit comprises a) a composition selected from the group consisting of a compound of Formula I, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising a compound of Formula I, and b) an instructional material.

15 The invention also includes a kit for inhibition of virus replication in a cell. The kit comprises a) a composition selected from the group consisting of a compound of Formula I, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising a compound of Formula I, and b) an instructional material.

The invention also includes a compound having the structure of Formula III:



20

(III)

wherein,

R^{11} is (C_1 - C_{16}) alkyl, branched alkyl, alkenyl or alkynyl;

R^{12} is (C_1 - C_{16}) alkyl, branched alkyl, alkenyl or alkynyl;

X^{11} is O, S, or $NHC=O$;

25

X^{12} is O, S, or $NHC=O$;

X^{13} is O or S;

n is 0, 1 or 2, and

R^{13} is a therapeutic agent,

wherein, each alkyl, branched alkyl, alkenyl, alkynyl, adenine, thymine,

5 cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^{11} , R^{12} , and R^{13} can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C_1 - C_8) alkyl, (C_1 - C_8) alkoxy, aryl, and $N(R^a)(R^b)$ wherein R^a and R^b are each independently selected from the group consisting of H and (C_1 - C_8) alkyl, and

10 wherein, if n is 1 or 2, the compound is a phospholipase C substrate and is not a phospholipase A substrate, and

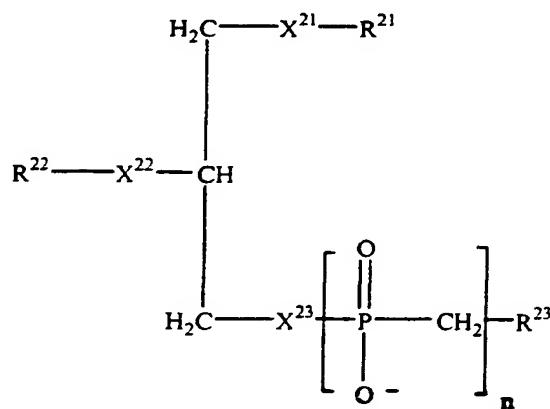
further wherein, if n is 1 or 2, the compound is converted to an alkyl lipid and a moiety selected from the group consisting of a nucleoside monophosphate and a nucleoside analogue monophosphate intracellularly in a mammal, and is not converted to an alkyl lipid and

15 a moiety selected from the group consisting of a nucleoside monophosphate and a nucleoside analogue monophosphate extracellularly in a mammal.

In a preferred embodiment, R^{11} is a C_{12} alkyl, branched alkyl, alkenyl or alkynyl; R^{12} is C_8H_{16} alkyl or branched alkyl; n = 1, and R^{13} is an anticancer agent selected from the group consisting of gemcitabine, ara-C, 5-azacytidine, cladribine, flucarabine,

20 fluorodeoxyuridine, cytosine arabinoside and 6-mercaptopurine, wherein the phosphorus atom of the phosphate moiety is covalently linked in a phosphate ester linkage to the oxygen atom of the 5' hydroxyl group of a sugar moiety of R^{13} .

The invention also includes a compound having the structure of Formula IV:



wherein,

R^{21} is (C_6 to C_{16}) alkyl, branched alkyl, alkenyl, or alkynyl;

R^{22} is (C_1 to C_{12}) alkyl, branched alkyl, alkenyl, or alkynyl;

X^{21} is O, S, or $NHC=O$;

5 X^{22} is O, S, or $NHC=O$;

X^{23} is O or S;

n is 1 or 2;

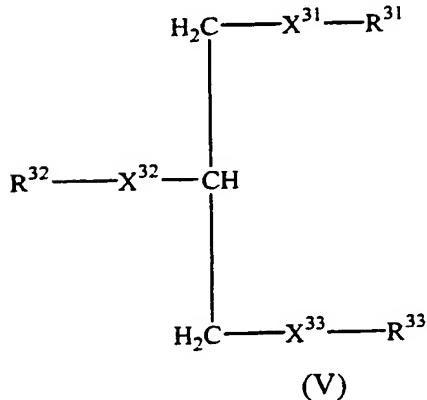
R^{23} is a therapeutic agent, and

wherein, each alkyl, branched alkyl, alkenyl, alkynyl, adenine, thymine,

10 cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^{21} , R^{22} , and R^{23} can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C_1 - C_8) alkyl, (C_1 - C_8) alkoxy, aryl, and $N(R^a)(R^b)$ wherein R^a and R^b are each independently selected from the group consisting of H and (C_1 - C_8) alkyl.

15 In a preferred aspect, R^{21} is C_{12} alkyl; R^{22} is C_{10} alkyl; n = 1, and R^{23} is an anticancer agent selected from the group consisting of gemcitabine, ara-C, 5-azacytidine, cladribine, flucarabine, fluorodeoxyuridine, cytosine arabinoside and 6-mercaptopurine, wherein the methylene group of the phosphonate moiety is covalently linked to the oxygen atom of the 5' hydroxyl group of a sugar moiety of R^{23} .

20 The invention also includes a compound having the structure of Formula V:



wherein,

R^{31} is (C_1 to C_{16}) alkyl, branched alkyl, alkenyl, or alkynyl;

25 R^{32} is (C_1 to C_{16}) alkyl, branched alkyl, alkenyl, or alkynyl;

X^{31} is O, S, or NHC=O;

X^{32} is O, S, or NHC=O;

X^{33} is $-\text{OH}$, $-\text{SH}$, or amino;

R³³ is a therapeutic agent, and

In a preferred embodiment, R³¹ is (C₆ –C₁₆) alkyl, branched alkyl, alkenyl or alkynyl; R³² is (C₁ –C₈) alkyl, branched alkyl, alkenyl or alkynyl, and R³³ is an anticancer agent selected from the group consisting of mitoxanthrone, methotrexate and CPT-11, and is covalently linked via an ester, amido or carbamate linkage to the –SH, OH or amino group of X³³.

In one aspect, the compound is suspended in a pharmaceutically acceptable carrier and is present in an amount effective to combat a cancer in a mammal.

Preferably, the cancer is a cancer selected from the group consisting of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma and a solid tumor.

20 In one aspect, the compound is present in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

Preferably, the therapeutic agent is an anticancer agent.

Preferably, the cell is in a mammal.

25 Preferably, the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

In one aspect, the CNS cell is an astrocyte or a glial cell.

In one embodiment, the compound is in the form of a pharmaceutically acceptable salt.

30 In one aspect, the compound is present in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

In one embodiment, the cell is in a mammal.

Preferably, the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

In one aspect, the compound is present in an amount effective to combat a cancer in a mammal.

5 In one embodiment, the compound in the pharmaceutically acceptable salt is present in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

Preferably, the therapeutic agent is an anticancer agent.

In one aspect, the cell is in a mammal.

10 In one embodiment, the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

In one aspect, the compound is present in an amount effective to combat a cancer in a mammal.

15 The invention also includes a drug delivery agent comprising a pharmaceutical composition. The composition comprises a compound of Formula III or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

In one aspect, the therapeutic agent is an anticancer agent.

In another aspect, the cell is in a mammal.

20 Preferably, the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

The invention also includes a drug delivery agent comprising a pharmaceutical composition. The composition comprises a compound of Formula III or a pharmaceutically acceptable salt thereof, in an amount effective to combat a cancer in a mammal.

25 Preferably, the cancer is a cancer selected from the group consisting of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma and a solid tumor.

The invention also includes a method of facilitating delivery of a therapeutic agent to a mammalian cell. The method comprises administering to the cell a pharmaceutical composition comprising a compound of Formula III or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of the therapeutic agent to the cell.

30 In one aspect, the therapeutic agent is an anticancer agent.

In another aspect, the cell is in a mammal.

Preferably, the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

The invention also includes a method of facilitating delivery of a therapeutic agent to a cell. The method comprises administering to the cell a pharmaceutical composition comprising a compound of Formula III or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of the therapeutic agent to the cell.

In one aspect, the cell is in a mammal.

In another aspect, the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

10 The invention also includes a method of combating a cancer in a mammal. The method comprises administering to the mammal a pharmaceutical composition comprising a compound of Formula III or a pharmaceutically acceptable salt thereof, in an amount effective to combat a cancer in the mammal.

15 In one aspect, the cancer is a cancer selected from the group consisting of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma and a solid tumor.

The invention also includes a method of treating a disease in a mammal. The method comprises administering to the mammal a pharmaceutical composition comprising a compound of Formula III, or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of a therapeutic agent to a cell in the mammal, thereby treating the disease.

20 In one aspect, the disease is a disease selected from the group consisting of a brain disease, a CNS disease, a lymphatic system disease, a reproductive system disease, a cardiovascular disease, a kidney disease and a liver disease.

25 The invention also includes a kit for combating a cancer in a mammal. The kit comprises a) a composition selected from the group consisting of a compound of Formula III, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising a compound of Formula III, and b) an instructional material.

The invention also includes a kit for facilitating delivery of a therapeutic agent to a mammalian cell. The kit comprises a) a composition selected from the group consisting of a compound of Formula III, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising a compound of Formula III, and
30 b) an instructional material.

Preferably, the therapeutic agent is an anticancer agent.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings.

Figure 1, comprising Figures 1A, 1B, 1C and 1D is a series of formulae depicting the chemical structures of several anticancer agents. Figure 1A depicts the chemical structure of BM21.1290. Figure 1B depicts the chemical structure of gemcitabine. Figure 1C depicts the chemical structure of ara-C. Figure 1D depicts the chemical structure of 5-azacytidine.

Figure 2 is a reaction scheme depicting the synthesis method for preparing a lipid backbone (i.e. an alkyl lipid) for the compounds of the invention.

Figure 3 is a reaction scheme depicting the synthesis method for preparing an AZT-malonic acid (AZT-MA) compound, which is an intermediate compound in the synthesis of the double targeting PC lipid-AZT conjugate compounds of the invention.

Figure 4 is a reaction scheme depicting the synthesis method for preparing a double targeting PC lipid-AZT conjugate compound of the invention (INK-20).

Figure 5 is a graph depicting the concentrations of radiolabeled BM21.1290 in plasma and various lymphoid tissue samples after administration of radiolabeled BM21.1290 to female C57BI/6 mice for a two week period. The data indicate the preferential uptake of BM21.1290 into lymphoid tissue of mice.

Figure 6 is a graph depicting the concentrations of radiolabeled BM21.1290 in plasma and brain tissue samples after administration of different doses of radiolabeled BM21.1290 to female C57BI/6 mice for a two week period. The data indicate that the concentration of BM21.1290 in brain tissue samples is equivalent to the concentration of the compound in plasma, indicating that the compound effectively crosses the blood-brain barrier in mice.

Figure 7, comprising Figures 7A and 7B, is a pair of formulae depicting the chemical structures of exemplary compounds of Formula III.

Figure 8, comprising Figures 8A and 8B, is a pair of formulae depicting the chemical structures of exemplary compounds of Formula IV.

Figure 9 is a formula depicting the chemical structure of an exemplary compound of Formula V.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods and compositions useful in drug delivery for treatment of a virus infection in a mammal by targeting the virus at two or more stages of the virus life cycle and thereby inhibiting virus replication. This mode of use of 5 antiviral compositions is referred to herein as double-targeting a virus infection. The compositions of the invention include compounds comprising at least two chemically combined (e.g. covalently conjugated) antiviral agents which have different modes of action. Because the antiviral agents have different modes of action, they target the virus life cycle at two or more different stages. By way of example and not by limitation, the compositions of the invention 10 include compounds having a nucleoside analogue or protease inhibitor moiety conjugated with a phosphocholine lipid (PC lipid) moiety. Also by way of example and not by limitation, the targets in the viral life cycle of the compounds of the invention may include stages involving reverse transcription, protease activity, and virus assembly. The methods and compositions of the invention are particularly useful in combating drug-resistant mutants of viruses because 15 viruses resistant to nucleoside analogues and protease inhibitors are still sensitive to inhibition by phospholipids.

As used herein, the term "conjugated with" means covalently attached to the same molecule.

The targeted virus may be any type of virus, and non-limiting exemplary viruses 20 include HIV-1, HIV-2, hepatitis virus (e.g. hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E viruses), and herpesviruses (e.g. herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, Epstein Barr virus, and human herpes viruses types 6, 7, and 8).

The compounds of the invention exhibit biological properties which are superior 25 to currently available antiviral drugs, including (i) reduced cytotoxicity accompanied by the ability of the mammal to tolerate a higher dose of the drug compared with nucleoside analogues or protease inhibitors alone, (ii) ability to target multiple distinct stages of virus replication (e.g. reverse transcription, protease processing of viral proteins and virus assembly, leading to non-replication or to production of defective progeny virus) (iii) ability to simultaneously deliver constant amounts of multiple antiviral agents (e.g. phosphocholine lipid and a nucleoside 30 analogue or phosphocholine lipid and a protease inhibitor) to virus-infected cells with **preferential uptake into the CNS, lymphoid tissues, and male and female genital tracts,** (iv) intracellular metabolism of the conjugate compound and simultaneous release of two antiviral

agents in cells in which virus is multiplying, (v) increased half-life of the compound *in vivo* compared with nucleoside analogues or protease inhibitors alone, (vi) prolonged duration of biological effect, presumably owing to protection of nucleoside analogue from rapid glucuronide formation in the liver of intact animals, and (vii) capacity to conjugate other small 5 molecular weight compounds to the phosphocholine (PC) lipid backbone for treatment of other diseases of the central nervous system (e.g. Alzheimer's, cancer), in addition to diseases such as AIDS, resulting from virus infection.

Previous studies have established that a PC moiety is an essential component for a phospholipid to exhibit optimal antiviral activity (Piantadosi et al., 1991, *J. Med. Chem.* 10 34:1408-1414; Krugner-Higby et al., 1995, *AIDS Res. & Human Retrovir.* 11:705-712). Compounds comprising phosphatidic acid, phosphoethanolamine, phosphoalkylpyridine, alcohol, or quaternary amine salt moieties were less active, more toxic, exhibited much lower differential selectivities, or some combination of these, relative to the corresponding PC lipids. In certain preferred compounds of the invention, a PC moiety is incorporated into the lipid 15 backbone to result in compounds which exhibit optimal antiviral activity, can accumulate into lymphoid tissues, testes, and vaginal secretions, and can pass the blood-brain barrier into the CNS. These anatomical sites serve as important reservoirs of virus during infection by viruses such as HIV-1, and also serve as sources of transmission of drug-resistant mutants.

The invention also includes methods of treating a virus infection in a cell or in a 20 mammal, such as a human, comprising administering to the cell or mammal a compound of the invention in an amount effective to alleviate or eliminate the virus infection or to alleviate a symptom associated with the infection.

The present invention also includes methods and compositions useful in drug delivery for facilitating delivery of a therapeutic agent to a mammalian cell. As used herein, 25 the term "facilitating delivery" or "to facilitate delivery" of a therapeutic agent to a mammalian cell means enhancing the uptake of a therapeutic agent in a mammalian cell to a level higher than the level of uptake of the therapeutic agent in an otherwise identical mammalian cell which is not administered a compound or composition of the invention. The uptake of a therapeutic agent can be enhanced, by way of example and not by limitation, by any one or more of the 30 following means: by bypassing the requirement for a cellular active transport mechanism for uptake of the therapeutic agent into a cell; by providing the therapeutic agent (i.e. a drug) intracellularly in an activated form, (i.e. the monophosphorylated form in the case of a

nucleoside analogue anticancer drug) thereby bypassing the requirement for intracellular activation of the therapeutic agent by an enzyme such as an intracellular kinase; by overcoming a physiological barrier to uptake of the therapeutic agent in a desired cell, such as low solubility, poor absorption from the stomach or small intestine, or impermeability to the blood-
5 brain barrier, to enable delivery of the therapeutic agent to sites not normally accessible thereto (i.e. CNS and lymphoid tissues).

The present invention also includes methods and compositions useful in drug delivery for combating a cancer in a mammal or for treating or alleviating a disease in a mammal.

10 As used herein, the term "combating a cancer" or "to combat a cancer" in a mammal means, for example, any one or more of the following: to increase survival of a mammal, to decrease or arrest tumor size in a mammal, or to increase the time period of remission of cancer regrowth in a mammal, relative to an otherwise identical mammal which was not administered a composition or compound of the invention.

15 As used herein, the term "therapeutic agent" means any compound or composition, which, upon entering a mammalian cell, is capable of being of benefit in alleviating or treating a disease in a mammal. By way of example and not by limitation, such compounds and compositions include small organic molecules, peptides, nucleoside analogues, anticancer agents, antiviral agents, ribozymes, antisense oligonucleotides and other drugs. The
20 disease may be any disease experienced by a mammal. By way of example and not by limitation, such diseases include diseases of the brain, CNS, lymphatic system, reproductive system, cardiovascular system, renal system and liver, among others.

As used herein, "alleviating a disease" means reducing the severity of a symptom of the disease.

25 As used herein, "treating a disease" means reducing the frequency with which a symptom of the disease is experienced by a mammal.

As used herein, the term "anticancer agent" means a therapeutic agent which is capable of exhibiting efficacy at combating a cancer in a mammal or in a mammalian cell, or any compound which is capable of being converted intracellularly to a compound which is
30 capable of exhibiting efficacy at combating a cancer in a mammal or in a mammalian cell.

The mammalian cell can be any type of mammalian cell, including both cancerous and non-cancerous cells. Examples of preferred cells include, but are not limited to,

CNS and lymphoid cells. Preferred lymphoid cells include lymphoma, spleen and thymus cells. Preferred CNS cells include brain cells, astrocytes, and glial cells. The cancer can be any type of cancer in a mammal. Preferably, the cancer is one or more of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma and a solid tumor.

5 The compositions of the invention include compounds which comprise an alkyl lipid or a phospholipid moiety covalently conjugated with a therapeutic agent. As used herein, the term "alkyl lipid" means that portion of any of the compounds of Formulas III, IV and V as described herein without the therapeutic agent moiety.

10 The invention also includes pharmaceutical compositions and kits for combating a cancer and/or for facilitating delivery of a therapeutic agent to a mammalian cell.

The invention also includes methods which comprise administering a compound of the invention, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the invention, in an amount effective to combat a cancer or in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

15 As used herein, the following terms are defined as follows, unless otherwise described: halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, alkylene, etc. denote both straight and branched groups; but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to.

20 The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

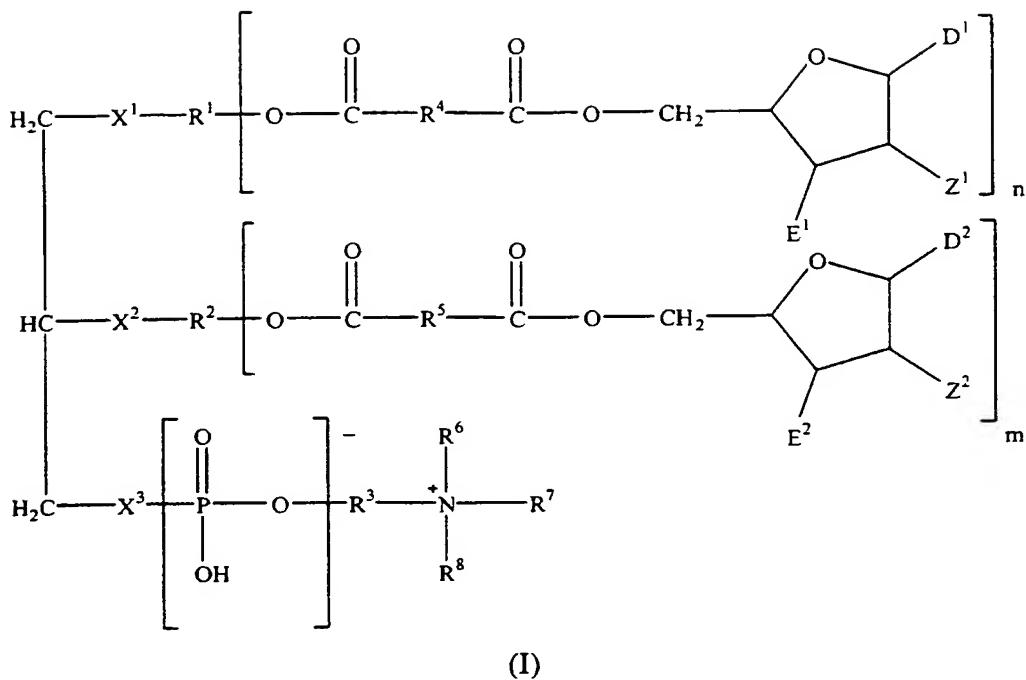
25 Compounds of the invention having a chiral center can exist in and be isolated in distinct optically active or racemic forms. The present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures of such forms of a compound of the invention. Preparation of optically active forms of a compound is well known in the art (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase). Determination or assessment of antiviral activity 30 can be performed using standard tests described herein or other tests known in the art.

Specific and preferred definitions listed below for radicals and substituents are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents described herein.

C_1 - C_8 alkyl moieties include, for example, methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, sec-pentyl, iso-pentyl, hexyl, sec-hexyl, iso-hexyl, heptyl, sec-heptyl, iso-heptyl, and octyl moieties. C_1 - C_8 alkoxy moieties include, for example, methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, sec-pentoxy, iso-pentoxy, hexyloxy, sec-hexyloxy, heptoxy, sec-heptoxy, iso-heptoxy, and octyloxy moieties. C_1 - C_8 alkylene moieties include, for example, methylene, ethylene, propylene, isopropylene, butylene, iso-butylene, sec-butylene, pentylene, sec-pentylene, iso-pentylene, hexylene, sec-hexylene, iso-hexylene, heptylene, sec-heptylene, iso-heptylene, and octylene moieties. C_6 - C_{15} alkyl moieties include, for example, hexyl, heptyl, sec-heptyl, iso-heptyl, octyl, sec-octyl, iso-octyl, nonyl, sec-nonyl, iso-nonyl, decyl, sec-decyl, iso-decyl, undecyl, sec-undecyl, iso-undecyl, dodecyl, sec-dodecyl, iso-dodecyl, tridecyl, sec-tridecyl, iso-tridecyl, tetradecyl, sec-tetradecyl, iso-tetradecyl, and pentadecyl moieties. C_6 - C_{15} alkylene moieties include, for example, hexylene, heptylene, sec-heptylene, iso-heptylene, octylene, sec-octylene, iso-octylene, nonylene, sec-nonylene, iso-nonylene, decylene, sec-decylene, iso-decylene, undecylene, sec-undecylene, iso-undecylene, dodecylene, sec-dodecylene, iso-dodecylene, tridecylene, sec-tridecylene, iso-tridecylene, tetradecylene, sec-tetradecylene, iso-tetradecylene, and pentadecylene moieties. C_8 - C_{12} alkyl moieties include, for example, octyl, sec-octyl, iso-octyl, nonyl, sec-nonyl, iso-nonyl, decyl, sec-decyl, iso-decyl, undecyl, sec-undecyl, iso-undecyl, and dodecyl moieties. C_8 - C_{12} alkylene moieties include, for example, octylene, sec-octylene, iso-octylene, nonylene, sec-nonylene, iso-nonylene, decylene, sec-decylene, iso-decylene, undecylene, sec-undecylene, iso-undecylene, and dodecylene moieties.

The present invention includes compounds which exhibit antiviral activity and are particularly useful because they exhibit antiviral activity against drug-resistant viruses. Accordingly, the invention includes a compound having the chemical structure of Formula I or a pharmaceutically acceptable salt thereof.

Formula I is



wherein

n and m are each independently 0 or 1, but n and m are not both 0;

5 R¹ is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl if n is 0 and (C₁-C₁₆) alkylene, alkenyl or alkynyl if n is 1;

R² is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl if m is 0 and (C₁-C₁₆) alkylene, alkenyl or alkynyl if m is 1;

R³, R⁴ and R⁵ are each independently (C₁-C₈) alkylene;

10 R⁶, R⁷ and R⁸ are each independently (C₁-C₈) alkyl;

X¹ and X² are each independently S, O, NHC=O, OC=O or NH;

X³ is O or S;

E¹ is H, S, halo or N₃;

Z¹ is H, S, or halo; or E¹ and Z¹ together are a covalent bond;

15 E² is H, S, halo, or N₃;

Z² is H, S, or halo; or E² and Z² together are a covalent bond, and

D¹ and D² are each independently selected from the group consisting of purine, pyrimidine, adenine, thymine, cytosine, guanine, hypoxanthine, inosine, uracil and ring modifications thereof, including O, N, and S substitutions.

In Formula I, each alkyl, alkylene, branched alkyl, alkenyl, alkynyl, adenine, thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, D¹, and D² can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoro, trifluoromethyl, 5 trifluoromethoxy, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

The following are examples of definitions for radicals and substituents of Formula I in preferred embodiments. These examples are not limiting, but are instead provided as examples of several preferred embodiments which are included in the invention.

10 In preferred embodiments, R¹ can be one of (C₂-C₁₆) alkylene, -(CH₂)₁₂-, and -CH=CH-. In these embodiments, R¹ is optionally substituted with 1, 2, 3, or 4 substituents selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

15 In preferred embodiments, R² can be one of (C₂-C₁₆) alkylene, -(CH₂)₈-, -(CH₂)₉-, -(CH₂)₁₀-, -(CH₂)₁₁-, -(CH₂)₁₂-, and -CH=CH-. In these embodiments, R² is optionally substituted with 1, 2, 3 or 4 substituents selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

20 R³ is preferably -CH₂CH₂-, optionally substituted with 1, 2, 3, or 4 substituents selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

25 R⁴ is preferably -CH₂- , optionally substituted with 1 or 2, substituents selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

30 R⁵ is preferably -CH₂-, optionally substituted with 1 or 2 substituents selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

In one preferred embodiment, each of R⁶, R⁷ and R⁸ is -CH₃, each optionally substituted with 1 or 2 substituents selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

5 X¹ is preferably S, O or NHC=O.

 X² is preferably S, O or NHC=O.

 X³ is preferably O or S.

 E¹ is preferably N₃, S, or H.

 Z¹ is preferably H.

10 E² is preferably N₃, S or H.

 Z² is preferably H.

 D¹ is preferably cytosine, guanine, inosine or thymine, wherein D₁ is optionally substituted with 1, 2, 3, or 4 substituents selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

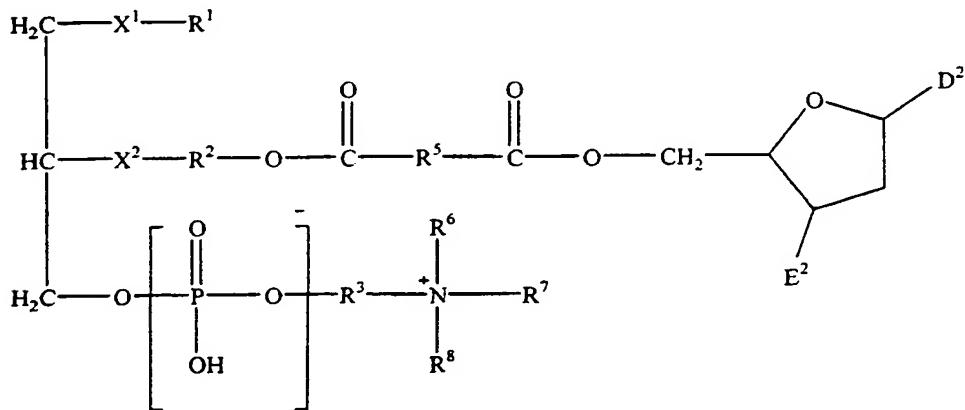
15

 D² is preferably cytosine, guanine, inosine or thymine, wherein D₂ is optionally substituted with 1, 2, 3 or 4 substituents selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

20

 The invention also includes a compound which exhibits antiviral activity having the chemical structure of Formula II or a pharmaceutically acceptable salt thereof.

Formula II is



wherein,

R¹ is (C₆-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl;

R² is (C₄-C₁₂) alkylene;

R³ is -CH₂CH₂-;

R⁵ is -CH₂-;

5 R⁶, R⁷ and R⁸ are each CH₃;

X¹ and X² are each independently S, O or NHC=O;

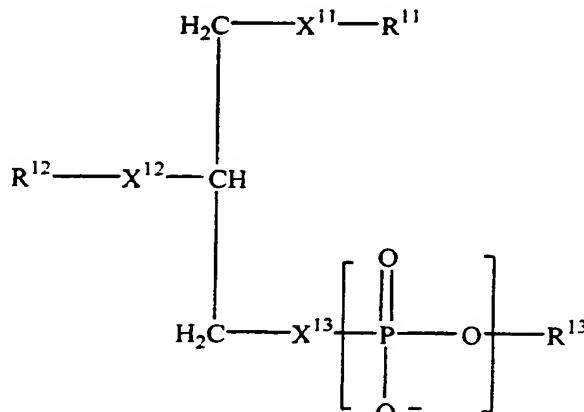
E² is H or N₃, and

D² is selected from the group consisting of thymine, cytosine, guanine and inosine.

10 In Formula II, each alkyl, branched alkyl, alkenyl, alkynyl, thymine, cytosine, guanine, and inosine of R¹, R², R³, R⁵, R⁶, R⁷, R⁸, and D² can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b), wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

15 The present invention also includes compounds which are useful in drug delivery for treating or alleviating a disease or combating a cancer in a mammal. The compounds are also useful for facilitating delivery of a therapeutic agent to a mammalian cell. Accordingly, the invention includes a compound having the chemical structure of Formula III or a pharmaceutically acceptable salt thereof.

20 Formula III is



(III)

wherein,

R^{11} is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl;

R^{12} is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl;

X^{11} is O, S, or NHC=O;

X^{12} is O, S, or NHC=O;

5 X^{13} is O or S;

n is 0, 1 or 2, and

R^{13} is a therapeutic agent.

In Formula III, each alkyl, branched alkyl, alkenyl, alkynyl, adenine, thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^{11} , R^{12} , and R^{13} can, 10 optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

15 In Formula III, if n is 1 or 2, the compound is a phospholipase C substrate and is not a phospholipase A substrate. Also, if n is 1 or 2, the compound is converted to an alkyl lipid and a moiety selected from the group consisting of a nucleoside monophosphate and a nucleoside analogue monophosphate intracellularly in a mammal, and is not converted to an alkyl lipid and a moiety selected from the group consisting of a nucleoside monophosphate and a nucleoside analogue monophosphate extracellularly in a mammal.

20 The conjugate compounds of Formula III can be formulated in pharmaceutical compositions as described herein, which have the advantageous properties of being suitable for oral administration, can be readily absorbed from the gastrointestinal tract, can cross the blood-brain barrier and be of value in the treatment of CNS diseases and cancers. These conjugates can be used in a number of different cell lines including, by way of example and not by limitation, brain tumor cells, lymphoid cells and pancreatic tumor cells.

25 In compounds of Formula III which have at least one phosphate group (i.e., n = 1 or 2) the phosphate ester linkage is cleaved intracellularly in a mammal by the action of a phospholipase C-like activity to release intracellularly a phospholipid and an anticancer agent. These compounds are substrates of phospholipase C, but not substrates of phospholipase A. 30 Because the phospholipase C activity is intracellular, the conjugates are only converted to a phospholipid and a nucleoside monophosphate intracellularly, and not extracellularly. The metabolism of these compounds by an intracellular phospholipase C-like activity enables the

compounds to be used in methods which circumvent the rate limiting step for the activation of nucleoside analogue prodrugs, namely, the conversion of nucleoside analogue to nucleoside analog monophosphate. Because they are metabolized intracellularly to release a nucleoside analogue monophosphate, the administration of these compounds results in the ability to

5 provide an anticancer agent which can be effective in cancer cells which lack a kinase enzyme such as, for example, deoxycytidine kinase, as a mechanism of cellular anticancer drug resistance. Additionally, the phospholipid moiety can affect signal transduction pathways involving protein kinase C and MAP kinase signaling cascades.

The released nucleoside monophosphate serves two purposes. First, it bypasses
10 the rate limiting step in the activation of several nucleoside prodrugs, namely, deoxycytidine kinase. Second, the polar phosphate group "locks" the nucleoside within the cell. The phospholipid conjugate also serves as a reservoir for the drug, increasing the drugs half-life. The capacity to conjugate other small molecular weight compounds to the phospholipid backbone for the treatment of other diseases of the central nervous system (i.e. Alzheimer's) is
15 also of great utility. For example, an ether-lipid moiety can be used as a backbone for conjugation to a variety of therapeutic agents including nucleoside analogues, anticancer and antiviral agents, ribozymes and antisense oligonucleotides. Since the ether-lipid backbone is lipophilic, these conjugates can cross the blood-brain barrier and be used as prodrugs in the treatment of CNS diseases, such as Alzheimer's and neurologic degenerative diseases. The
20 lipophilic property of the conjugates enables them to cross the blood-brain barrier, and thus bypass the requirement for an active transport system in the cell in which uptake of the drug is desired.

In preferred compounds of Formula III,

25 R^{11} is a C_{12} alkyl, branched alkyl, alkenyl or alkynyl;

R^{12} is C_8H_{16} alkyl or branched alkyl;

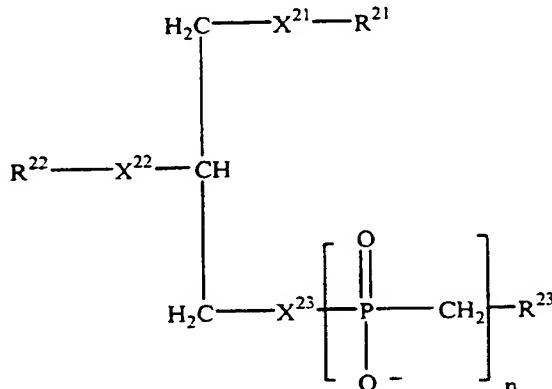
$n = 1$,

and R^{13} is an anticancer agent.

Preferably, the anticancer agent is selected from the group consisting of gemcitabine, ara-C, 5-azacytidine, cladribine, flucytosine, fluorodeoxyuridine, cytosine
30 arabinoside and 6-mercaptopurine, wherein the phosphorus atom of the phosphate moiety is covalently linked in a phosphate ester linkage to the oxygen atom of the 5' hydroxyl group of a sugar moiety of R^{13} .

The invention includes additional compounds which are useful in drug delivery for treating or alleviating a disease or combating a cancer in a mammal. The compounds are also useful for facilitating delivery of a therapeutic agent to a mammalian cell. Accordingly, the invention includes a compound having the chemical structure of Formula IV or a 5 pharmaceutically acceptable salt thereof.

Formula IV is



(IV)

wherein,

10 R^{21} is (C_6 to C_{16}) alkyl, branched alkyl, alkenyl, or alkynyl;

R^{22} is (C_1 to C_{12}) alkyl, branched alkyl, alkenyl, or alkynyl;

X^{21} is O, S, or $\text{NHC}=\text{O}$;

X^{22} is O, S, or $\text{NHC}=\text{O}$;

X^{23} is O or S;

15 n is 1 or 2, and

R^{23} is a therapeutic agent.

In Formula IV, each alkyl, branched alkyl, alkenyl, alkynyl, adenine, thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^{21} , R^{22} , and R^{23} can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group 20 consisting of halo, nitro, trifluoromethyl, (C_1 - C_8) alkyl, (C_1 - C_8) alkoxy, aryl, and $\text{N}(\text{R}^a)(\text{R}^b)$ wherein R^a and R^b are each independently selected from the group consisting of H and (C_1 - C_8) alkyl.

In preferred compounds of Formula IV,

R^{21} is C_{12} alkyl;

25 R^{22} is C_{10} alkyl;

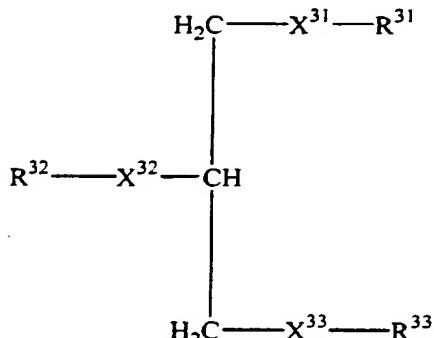
n = 1, and

R²³ is an anticancer agent.

Preferably, the anticancer agent is selected from the group consisting of gemcitabine, ara-C, 5-azacytidine, cladribine, flucytosine, fluorodeoxyuridine, cytosine 5 arabinoside and 6-mercaptopurine, wherein the methylene group of the phosphonate moiety is covalently linked to the oxygen atom of the 5' hydroxyl group of a sugar moiety of R²³.

The invention includes additional compounds which are useful in drug delivery for treating or alleviating a disease or combating a cancer in a mammal. The compounds are also useful for facilitating delivery of a therapeutic agent to a mammalian cell. Accordingly, 10 the invention includes a compound having the chemical structure of Formula V or a pharmaceutically acceptable salt thereof.

Formula V is



15

(V)

wherein,

R³¹ is (C₁ to C₁₆) alkyl, branched alkyl, alkenyl, or alkynyl;

R³² is (C₁ to C₁₆) alkyl, branched alkyl, alkenyl, or alkynyl;

X³¹ is O, S, or NHC=O;

20 X³² is O, S, or NHC=O;

X³³ is -OH, -SH, or amino, and

R³³ is a therapeutic agent.

In Formula V, each alkyl, branched alkyl, alkenyl, alkynyl, adenine, thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R³¹, R³², and R³³ can, 25 optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b)

wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

In preferred compounds of Formula V,

R³¹ is (C₆-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl;

5 R³² is (C₁-C₈) alkyl, branched alkyl, alkenyl or alkynyl, and

R³³ is an anticancer agent.

Preferably, the anticancer agent is selected from the group consisting of mitoxanthrone, methotrexate and CPT-11, and is covalently linked via an ester, amido or carbamate linkage to the -SH, OH or amino group of X³³.

10 Compounds of Formula I and Formula II can be prepared according to procedures known to the skilled artisan (See, for example, Marx et al., 1988, Journal of Medicinal Chemistry 31:858-863; Meyer et al., 1991, Journal of Medicinal Chemistry 34:1377-1383; Morris-Natschke et al., 1986, Journal of Medicinal Chemistry 29:2114-2117; Piantadosi et al., 1991, Journal of Medicinal Chemistry 34:1408-1414; and Surles et al., 1993, Lipids 15 28:55-57).

An example of such a procedure is illustrated in Figures 2-4. The structures presented in the reaction schemes of Figures 2-4 are representative and not meant to limit the compounds of the invention. Modifications to the reactions in Figures 2-4 using different compounds are apparent to the skilled artisan. Briefly, a compound of Formula I or Formula II is prepared by reacting a lipid backbone moiety, prepared as shown in Figure 2, for example, with an AZT-malonic acid (AZT-MA) moiety, for example, prepared as shown in Figure 3. Synthetic methods for the preparation of a lipid backbone as described in Figure 2 are known in the art. For example, the synthesis method for preparing a lipid backbone for a thiophosphocholine is described in Morris-Natschke et al., 1986, Journal of Medicinal Chemistry 29(10):2114-2117, except one would substitute the benzyloxy alkyl bromide for the C-2 alkyl chain described in the reference. To prepare an amidophosphocholine, for example, one would follow the synthesis method described in Kucera et al., 1998, Antiviral Chemistry and Chemotherapy, 9:157-165. However, one would substitute C₆H₅CH₂O(CH₂)₈Br (8-benzyloxyoctyl bromide) for CH₃(CH₂)₇Br (octyl bromide) described in the reference. To 20 prepare a lipid backbone for various other phosphocholine syntheses, one would follow the synthesis procedures described in Meyer et al., 1991, Journal of Medicinal Chemistry 34(4):1377-1383 and Morris-Natschke et al., 1993, Journal of Medicinal Chemistry 36(14): 25 30

2018-2023. Again, one would substitute the benzyloxy alkyl bromide for the C-2 alkyl chain described in the references.

A preferred compound of the invention (e.g. INK-20, a PC lipid-AZT conjugate) can be prepared as described in the Examples herein and depicted in Figure 4 by reacting the 5 lipid backbone moiety generated as shown in Figure 2 with the AZT-malonic acid (AZT-MA) moiety generated as shown in Figure 3. The AZT-MA moiety can be prepared, for example, as described in the Examples herein. Figures 2-4 together illustrate the reaction scheme for preparation of certain preferred compounds of the invention, wherein AZT is linked to a PC lipid at the terminal functionality of position-2 on a modified thioglycerol backbone. The 10 intermediate thiophosphocholine in Figure 4 has a terminal hydroxyl group on the position-2 side chain which is used as a site for conjugating AZT to the PC lipid. An antiviral agent such as, for example, AZT or a protease inhibitor can be linked to the PC lipid via a malonic ester. This synthetic pathway allows manipulation of the rate of esterase-catalyzed hydrolysis of the 15 AZT moiety in the cell by incorporation of substituted malonic linking groups. While not wishing to be bound by any particular theory, it is expected that, as with accepted prodrug strategy, the ester bond linking the PC lipid with the AZT moiety is cleaved by the action of esterase-like activity *in vivo*, thereby releasing both active antiviral agents (e.g. nucleoside or protease inhibitor and PC lipid) inside treated cells (See Chapter 47, "Chemotherapy of 20 Microbial Agents," pp. 1130 and 1141, respectively, in Goodman and Gilman, 1996, "The Pharmacological Basis of Therapeutics", Ninth Ed.).

The following compounds are illustrative of compounds having structures according to one or both of Formula I and Formula II, as described above. These compounds can be prepared by the procedures described herein, or by variations thereof which are apparent to those skilled in the art in view of the instant disclosure. Exemplary compounds include INK- 25 20, INK-25 and INK-26. The chemical structures of these compounds are depicted in Table 1 herein.

Compounds of Formulae III, IV and V can be prepared according to procedures known to the skilled artisan. An example of such a procedure is described, for example, in Piantadosi et al., 1991, J. Med. Chem. 34:1408-1414. The synthesis of a compound of 30 Formula V involves direct esterification of the lipid portion with the therapeutic agent rather than conjugation of the therapeutic agent with the phosphatidic acid portion of Formulae III and IV.

Exemplary compounds having structures according to Formulae III, IV and V, are described herein in the Figures. These compounds can be prepared by the procedures described herein, or by variations thereof which are apparent to those skilled in the art in view of the instant disclosure. Structural formulae of exemplary compounds are shown in Figure 7 5 (Formula III), Figure 8 (Formula IV), and Figure 9 (Formula V).

The compounds of the present invention can be prepared in the form of a pharmaceutically acceptable salt or a non-pharmaceutically acceptable salt. Non-pharmaceutically acceptable salts are useful, for example, as intermediates for preparation of a pharmaceutically acceptable salt. When the compounds are sufficiently basic or acidic to form 10 stable non-toxic acid or base salts, the compounds may be prepared as a pharmaceutically acceptable salt. Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesirable toxicological effects.

Examples of such salts are acid addition salts formed with inorganic acids, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, and nitric acids and the like; salts 15 formed with organic acids such as acetic, oxalic, tartaric, succinic, maleic, fumaric, gluconic, citric, malic, methanesulfonic, p-toluenesulfonic, napthalenesulfonic, and polygalacturonic acids, and the like; salts formed from elemental anions such as chlorine, bromine, and iodine; salts formed from metal hydroxides, for example, sodium hydroxide, potassium hydroxide, calcium hydroxide, lithium hydroxide, and magnesium hydroxide; salts formed from metal 20 carbonates, for example, sodium carbonate, potassium carbonate, calcium carbonate, and magnesium carbonate; salts formed from metal bicarbonates, for example, sodium bicarbonate and potassium bicarbonate; salts formed from metal sulfates, for example, sodium sulfate and potassium sulfate; and salts formed from metal nitrates, for example, sodium nitrate and potassium nitrate.

25 Pharmaceutically acceptable and non-pharmaceutically acceptable salts may be prepared using procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid comprising a physiologically acceptable anion. Alkali metal (for example, sodium, potassium, or lithium) or alkaline earth metal (for example, calcium) salts of carboxylic acids can also be made.

30 The compounds of Formulae I - V can be formulated as pharmaceutical compositions and administered to a mammal, such as a human patient by a chosen route of administration. Pharmaceutical compositions that are useful in the methods of the invention

can be prepared, packaged, or sold in a variety of formulations which can be suitable for one or more routes of administration such as, for example, oral, intravenous, intramuscular, topical, subcutaneous, rectal, vaginal, parenteral, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. Other contemplated formulations include projected nanoparticles, 5 liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are 10 generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the 15 invention is contemplated include, but are not limited to, humans and other primates and mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

Thus, the present compounds can be systemically administered (e.g. orally) in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an 20 assimilable edible carrier. They can be enclosed in hard or soft shell gelatin capsules, compressed into tablets, or incorporated directly into the food of the patient's diet. For oral therapeutic administration, the active compound can be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1 % 25 (w/w) of active compound. The percentage of the compositions and preparations can, of course, be varied, for example from about 0.1 % to nearly 100 % of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained upon administration.

The tablets, troches, pills, capsules, and the like can also contain one or more of 30 the following: binders such as gum tragacanth, acacia, corn starch, or gelatin; excipients such as **dicalcium phosphate**; a disintegrating agent such as corn starch, potato starch, alginic acid, and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose,

fructose, lactose, or aspartame; and a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the unit dosage form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials can be present as coatings or to otherwise modify the physical form of 5 the solid unit dosage form. For instance, tablets, pills, or capsules can be coated with gelatin, wax, shellac, sugar, and the like. A syrup or elixir can contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye, and flavoring such as cherry or orange flavor. Of course, any material used in preparing a unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts 10 employed. In addition, the active compound can be incorporated into sustained-release preparations and devices.

The active compound can be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a non-toxic surfactant. Dispersions can be prepared in glycerol, liquid 15 polyethylene glycols, triacetin, mixtures thereof, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent growth of microorganisms.

Pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or 20 dispersions, optionally encapsulated in liposomes. The ultimate dosage form should be sterile, fluid, and stable under conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, 25 for example, by formation of liposomes, by the maintenance of the required particle size (in the case of dispersions) or by use of one or more surfactants. Microbial growth can be prevented using various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers, or sodium chloride. Prolonged absorption of the injectable 30 compositions can be achieved using agents which delay absorption, for example, aluminum **monostearate and gelatin.**

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in an appropriate solvent, optionally with one or more of the other ingredients enumerated above, followed by filter sterilization. In the case of sterile powders for preparation of sterile injectable solutions, preferred methods of preparation include vacuum 5 drying and the freeze drying techniques, which yield a powder of the active ingredient and any additional desired ingredient present in the previously sterile-filtered solution(s).

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or 10 colonic irrigation.

Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (i.e. about 20°C) and which is liquid at the rectal temperature of the subject (i.e. about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include, but 15 are not limited to, cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As 20 is well known in the art, enema preparations may be administered using, and may be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

A pharmaceutical composition of the invention may be prepared, packaged, or 25 sold in a formulation suitable for vaginal administration. Such a composition may be in the form of, for example, a suppository, an impregnated or coated vaginally-insertable material such as a tampon, a douche preparation, or a solution for vaginal irrigation.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a 30 chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e. such as with a physiologically

degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Douche preparations or solutions for vaginal irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, douche preparations may be administered using, and may be packaged within, a delivery device adapted to the vaginal anatomy of the subject. Douche preparations may further comprise various additional ingredients including, but not limited to, antioxidants, antibiotics, antifungal agents, and preservatives.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions preferably include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65°F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (preferably having a particle size of the same order as particles comprising the active ingredient).

Pharmaceutical compositions of the invention formulated for pulmonary delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be prepared, packaged, or sold as aqueous or dilute

alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active 5 agent, or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers.

The formulations described herein as being useful for pulmonary delivery are also useful for intranasal delivery of a pharmaceutical composition of the invention.

10 Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered in the manner in which snuff is taken i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

15 Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

20 A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1-1.0% (w/w) solution or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

25 For topical administration, the present compounds can be applied in pure form, i.e., as a liquid. However, it will generally be desirable to administer the compounds to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

30 Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina, and the like. Useful liquid carriers include water, alcohols, glycols, and blends of two or more of these, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants.

Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize properties for a given use. The resulting liquid compositions can be applied using absorbent pads, used to impregnate bandages or other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

5 Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses, or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

10 Examples of useful dermatological compositions which can be used to deliver the compounds of the invention to the skin are disclosed in Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

15 Accordingly, the invention includes pharmaceutical compositions comprising one or more compounds of Formula I, Formula II, Formula III, Formula IV or Formula V, or any combination thereof, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

20 In a preferred embodiment, the pharmaceutical composition is adapted for oral, topical, or parenteral administration to a mammal such as a human, and comprises one or more compounds of Formula I or Formula II, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to treat a virus infection in a mammal or in a cell, particularly wherein the virus is HIV, hepatitis virus, or herpes simplex virus.

25 As used herein, "treatment" of a virus infection can mean, for example, any one or more of the following: inhibiting the replication of the virus, reducing the virus load within a patient, inhibiting formation of infectious progeny virus, inhibiting infectiousness of virus, killing cells harboring virus, interfering with one or more stages of the virus life cycle, inhibiting one or more viral enzymes or inducing production of non-infectious virus particles that can activate an immune response against infectious virus (e.g. autovaccination).

30 In another preferred embodiment, the pharmaceutical composition is adapted for oral, topical, or parenteral administration to a mammal such as a human, and comprises one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to combat a cancer in a mammal and/or to facilitate delivery of a therapeutic agent to a mammalian cell.

Useful dosages of the compounds of Formulae I-V for inclusion in the pharmaceutical compositions of the invention can be determined by comparing *in vitro* activity and *in vivo* activity of the compounds in appropriate animal models. Methods for the extrapolation of effective dosages in mice and other animal models to humans are known in the 5 art (see, for example U.S. Pat. No. 4,938,949).

Generally, the concentration of the compound(s) of Formulae I-V in a liquid composition, such as a lotion, will range from about 0.1 % to about 95 % by weight, preferably from about 0.5 % to about 25 % by weight. The concentration in a semi-solid or solid composition such as a gel or a powder will range from about 0.1 % to 100% by weight, 10 preferably about 0.5 % to about 5 % by weight. Single doses for intravenous injection, subcutaneous, intramuscular or topical administration, infusion, ingestion or suppository will generally be from about 0.001 to about 5000 mg, and be administered from about 1 to about 3 times daily, to yield levels of about 0.01 to about 500 mg/kg, for adults.

The invention also includes one or more compounds of Formula I or Formula II, 15 or any combination thereof, in an amount effective to inhibit virus replication in a mammal. The compound of course is therefore useful for inhibiting virus replication in a cell or neutralization (i.e. inactivation) of extracellular virus. Additionally, the invention includes one or more pharmaceutically acceptable salts of a compound of Formula I or Formula II, or any combination thereof, wherein the compound is present in an amount effective to inhibit virus 20 replication in a mammal.

As used herein, to inhibit virus replication in a mammal means to reduce the virus load in a mammal to a level which is lower than the level of the virus load in an otherwise identical mammal which was not administered the compound. Preferably, virus load in a mammal is reduced by about 1 to 12 \log_{10} or more relative to an otherwise identical mammal 25 which was not administered the compound. Virus load in a mammal can be assessed by a number of methods known in the art such as, for example, obtaining a tissue or fluid sample from the mammal and assessing the amount of virus or viral components in the mammal contained therein using technology which is either virological, immunological, biochemical or molecular biological in nature and which is well known to the skilled artisan and which are 30 described elsewhere herein. Inhibition of virus replication in a cell is assessed using similar or identical assays as those used to assess virus load in a mammal.

The invention also includes a kit for administering a composition of the invention (e.g. a compound of the invention, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the invention) to a mammal for treatment of a virus infection. Preferably, the mammal is a human. The virus infection can be an infection by any one or more of the viruses described herein. The kit comprises the composition of the invention and an instructional material which describes adventitiously administering the composition to the mammal by any of the routes of administration described herein. In another embodiment, this kit comprises a (preferably sterile) solvent suitable for dissolving or suspending the composition of the invention prior to administering the compound to the mammal.

As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the composition of the invention in the kit for any one or more of the following: effecting treatment of a virus infection in a mammal or in a cell; alleviation or treatment of the symptoms of a virus infection in the mammal; combating a cancer in a mammal; or for facilitating delivery of an anticancer agent to a mammalian cell. The instructional material of the kit of the invention may, for example, be affixed to a container which contains the composition of the invention or be shipped together with a container which contains the composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the composition be used cooperatively by the recipient.

The invention also includes a kit for inhibition of virus replication in a cell. The kit comprises a composition of the invention, which can be one or more compounds of Formula I or Formula II, or any combination thereof, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising one or more compounds of Formula I or Formula II, or any combination thereof. The kit also includes an instructional material.

As used herein, inhibition of virus replication in a cell means a reduction in virus replication in a cell to a level lower than the level in an otherwise identical cell which was not administered the composition of the invention. Preferably, the reduction in virus replication is by about 90 to about 99.9 % relative to the otherwise identical cell which was not administered the composition of the invention. The level of virus replication in a cell and therefore virus load in a mammal that is also being assessed, can be assessed by any one of numerous methods known to the skilled artisan. For example, the level of virus replication in a cell can be

assessed by evaluating the number of virus particles or amount of a viral component, such as a viral protein, a viral enzyme, or viral nucleic acid, in the cell or in fluid or debris associated with the cell. The number of infectious virus particles in a cell can be evaluated, for example, in a plaque assay. The level of a viral component such as a viral protein or enzyme in a cell can 5 be evaluated using standard analytical techniques of protein biochemistry, such as, for example, using an activity assay for a viral enzyme, or using Western blotting or quantitative gel electrophoresis for a viral protein. Viral nucleic acid levels in a cell can be evaluated using standard analytical techniques such as Northern blotting and Southern Blotting or quantitation by polymerase chain reaction (PCR).

10 The invention includes methods for treatment of a virus infection in a mammal. The methods comprise administering to the mammal one or more compounds of Formula I or Formula II, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to treat the virus infection. The compound may be administered by any of the methods described herein. Preferably, the mammal is a human. The virus infection may be 15 caused by any type of virus. Preferably, the virus infection results from infection by a virus selected from the group consisting of HIV-1, HIV-2, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, herpes simplex virus type 1, herpes simplex virus type 2, varicella-zoster virus, cytomegalovirus, Epstein Barr virus, human herpes virus type 6, human herpes virus type 7 and human herpes virus type 8, parainfluenza viruses 20 and respiratory syncytial viruses.

The invention also includes methods of treating a virus infection in a mammal by contacting the virus *in vitro*, *in vivo* or *ex-vivo* with one or more compounds of Formula I or Formula II, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to treat the virus infection (e.g. to inhibit virus replication, infectivity, life 25 cycle processes or pathogenesis). Methods for testing the antiviral activity of a compound *in-vitro* are known to the skilled artisan, and are described, for example, in Kucera et al., 1990, AIDS Res. and Human Retrovir. 6:494.

30 The invention further includes methods of using one or more compounds of Formula I or Formula II, or any combination thereof, or a pharmaceutically acceptable salt thereof, in medical therapy (preferably for use in treating a virus infection) or for the **manufacture of a medicament useful for the treatment of a virus infection.**

The invention also includes methods of inhibiting virus replication in a cell. The methods comprise administering to the cell one or more compounds of Formula I or Formula II, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to inhibit virus replication in the cell. Inhibition of virus replication in a cell, as 5 described herein, means a reduction in virus replication in a cell to a level lower than the level in an otherwise identical cell which was not administered the compound of the invention. Preferably, the reduction in virus replication is by about 90 % to about 99.9 % relative to the otherwise identical cell which was not administered the compound of the invention.

The level of virus replication in a cell can be assessed by any one of the methods 10 known to the skilled artisan described herein.

The invention also includes one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof, or a pharmaceutically acceptable salt thereof, wherein the compound is present in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell. Preferably, the therapeutic agent is an anticancer agent as described 15 herein.

In a preferred embodiment, the compound is suspended in a pharmaceutically acceptable carrier and is present in an amount effective to facilitate delivery of an anticancer agent to a mammalian cell.

Preferably, the cell is in a mammal. Also, preferably, the cell is a cell selected 20 from the group consisting of a CNS cell and a lymphoid cell. Preferred CNS cells include an astrocyte and a glial cell.

Additionally, the invention includes one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof, or a pharmaceutically acceptable salt thereof, wherein the compound is present in an amount effective to combat a cancer in a 25 mammal.

Preferably, the cancer is one or more of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma, and a solid tumor.

In a preferred embodiment, the compound is suspended in a pharmaceutically acceptable carrier and is present in an amount effective to combat a cancer in a mammalian cell.

30 Preferably, the cell is in a mammal. Also, preferably, the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell. Preferred CNS cells include an astrocyte and a glial cell.

The invention also includes a drug delivery agent comprising a pharmaceutical composition. The pharmaceutical composition comprises one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell. Preferably, the therapeutic agent is an anticancer agent. Preferably, the cell is in a mammal. Also, preferably, the cell is one or more of a CNS cell and a lymphoid cell.

5 The invention also includes a drug delivery agent comprising a pharmaceutical composition. The pharmaceutical composition comprises one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to combat a cancer in a mammal. Preferably, the cancer is one or more of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma, and a solid tumor.

10 Additionally, the invention includes a method of facilitating delivery of a therapeutic agent to a cell. Preferably, the therapeutic agent is an anticancer agent. The method comprises administering to the cell a pharmaceutical composition comprising one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of the therapeutic agent to the cell. Preferably, the cell is in a mammal. A preferred cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

15 20 Further, the invention includes a method of combating a cancer in a mammal. The method comprises administering to the mammal a pharmaceutical composition comprising one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof, or a pharmaceutically acceptable salt thereof, in an amount effective to combat a cancer in the mammal. Preferably, the mammal is a human. A preferred cancer is a cancer selected from the group consisting of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma, and a solid tumor.

25 30 The invention also includes a method of treating or alleviating a disease in a mammal. The disease can be any disease experienced by a mammal. Preferably, the disease is one or more of a brain disease, a CNS disease, a lymphatic system disease, a reproductive system disease, a cardiovascular disease, a kidney disease and a liver disease. The method comprises administering to the mammal a pharmaceutical composition comprising a compound

of the invention, or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of a therapeutic agent to a cell in the mammal.

The invention also includes a kit for administering a composition of the invention (e.g. a compound of the invention, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the invention) to a mammal for combating a cancer in a mammal. Preferably, the mammal is a human. The cancer can be any of the types of cancer described herein. The kit comprises the composition of the invention and an instructional material which describes adventitiously administering the composition to the mammal by any of the routes of administration described herein. In another embodiment, this kit comprises a (preferably sterile) solvent suitable for dissolving or suspending the composition of the invention prior to administering the compound to the mammal.

The invention also includes a kit for facilitating delivery of a therapeutic agent to a mammalian cell. Preferably, the therapeutic agent is an anticancer agent. The kit comprises a composition of the invention, which can be one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising one or more compounds of Formula III, Formula IV or Formula V, or any combination thereof. The kit also includes an instructional material.

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention is not limited to these Examples, but rather includes all variations which are evident as a result of the teaching provided herein.

Example 1

Anti-HIV-1 Activity of Double-Targeting PC lipid-AZT Conjugate Compounds

The inhibitory effects of PC lipids, non-PC lipid-AZT conjugates, and double-targeting PC lipid-AZT conjugate compounds of the invention on replication of HIV-1 virus in cells was examined using the plaque assay procedure described in Kucera et al. (1990, AIDS Research and Human Retroviruses, 6:491). CEM-SS cells were seeded at 50,000 cells per milliliter in RPMI growth medium as a monolayer in 96-well dishes, inoculated with 50 to 100 plaque forming units of HIV-1 and overlaid with serial dilutions of either PC lipid, non-PC lipid-AZT conjugate, or PC-lipid-AZT conjugate in RPMI-1640 growth medium supplemented with 10% fetal bovine serum. The structures of the tested compounds are described in Table 1.

AZT and PC lipids were used as positive controls. Plaques were counted after incubating the dishes for five days at 37°C to determine the 50% effective concentration (EC₅₀) for the compounds tested. The cytotoxicity of PC lipids, non-PC lipid-AZT conjugates and double targeting PC lipid-AZT conjugates was assessed using the procedure described in Kucera et al.(1990, AIDS Res. And Human Retrovir., 6:496, and 1998, Antiviral Chemistry and Chemotherapy, 9:160).

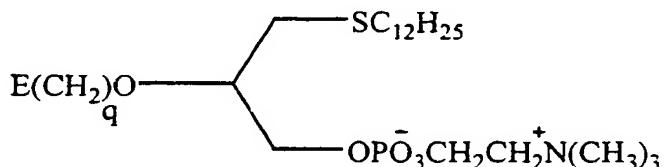
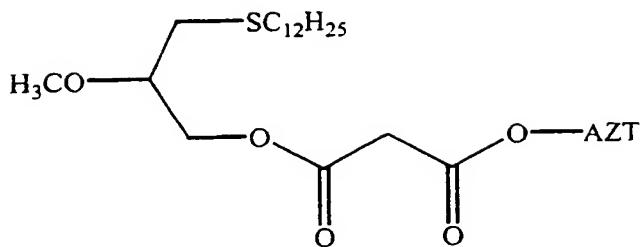


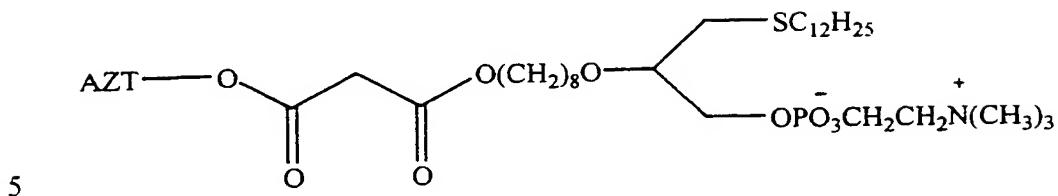
Table 1. (Formula VI)

Compound	Value of "q" in Formula VI	Identity of "E" in Formula VI
INK 17	8	-OCH ₂ C ₆ H ₅
INK-18	8	-OH
INK-19	See structure below	
INK-20	8	-O ₂ CCH ₂ CO ₂ AZT
INK-21	10	-OCH ₂ C ₆ H ₅
INK-22	10	-OH
INK-23	12	-OCH ₂ C ₆ H ₅
INK-24	12	-OH
INK-25	10	-O ₂ CCH ₂ CO ₂ AZT
INK-26	12	-O ₂ CCH ₂ CO ₂ AZT

10 The structure of INK-19 is



The structure of INK-20 is



In the plaque assay, HIV-1 syncytial plaques are seen as large, multicellular foci (10 to 25 nuclei/syncytium) that appear either brown and granular or clear. Because the 10 number of HIV-1 syncytial plaques was correlated with reverse transcriptase (RT) and p24 core antigen activity in the HIV-1 infected cell overlay fluids, the syncytial plaque assay could be used to quantify the amount of infectious virus. Reverse transcriptase activity was assayed according to the procedure described by Poeisz et al. (1980, Proc. Natl. Acad. Sci. U.S.A. 77:7415). The activity of p24 core antigen induced by HIV-1 infection of CEM-SS cells was measured spectrophotometrically using the commercial Coulter enzyme immunoassay (EIA).

15 The results of these assays are presented in Table 2. These results demonstrate that the double-targeting PC lipid-AZT conjugate compounds of the invention exhibit a higher differential selectivity (DS = ratio of TC₅₀ for cytotoxicity to EC₅₀ for anti-HIV activity) than the PC lipids, the non-PC lipid-AZT conjugates and the positive control, AZT. For example, the compound INK-20 exhibited the highest differential selectivity (DS = 7,666) and anti-HIV- 20 1 activity (0.0009 micromolar) of all the compounds tested, while exhibiting a cytotoxicity (6.9 ± 0.6 micromolar) comparable to AZT. The anti-HIV-1 activity of INK-20 was more than ten-fold higher than AZT.

Table 2.

Compound	Cytotoxicity (TC ₅₀) (μ M)	Anti-HIV-1 Activity (EC ₅₀) (μ M)	Differential Selectivity
INK-17 ^a	49.1 \pm 13.2	0.15 \pm 0.13	327
INK-18 ^a	50.0 \pm 42	1.92 \pm 1.8	26
INK-19 ^b	6.3 \pm 1.1	0.006 \pm 0.001	1050
INK-20 ^c	6.9 \pm 0.6	0.0009 \pm 0.00007	7666
INK-21 ^a	57.3 \pm 10.8	1.50 \pm 0.23	38
INK-22 ^a	> 90.5 \pm 13.4	0.39 \pm 0.31	> 232
INK-23 ^a	> 100.0 \pm 0.0	> 1.46 \pm 0.76	> 68
INK-24 ^a	80.6 \pm 6.2	0.39 \pm 0.39	207
INK-25 ^c	13.6	0.02 \pm 0.01	682
INK-26 ^c	11.6	0.02 \pm 0.02	580
BM21-1290 ^d	38.5	0.02	1925
AZT	3.7	0.009	411

^a PC-Lipid

^b Lipid ester linked-AZT (non-PC lipid-AZT conjugate)

^c A double-targeting compound of the invention (PC lipid-AZT conjugate)

^d A thioetherglycerolphospholipid-AZT conjugate (non-PC lipid-AZT conjugate) with AZT at position-3

10 Data indicating that PC lipids alone can inhibit infectious HIV-1 production, but not reverse transcriptase activity, in acutely infected human lymphocytes (CEM-SS) after 13 days treatment was previously published (Kucera et al., 1990, AIDS Research and Human Retroviruses, 6:497, Table 3). In contrast, the PC lipid-AZT conjugate compounds of the invention can inhibit both infectious HIV-1 production and reverse transcriptase activity in acutely infected human lymphocytes under similar test conditions. In summary, these data 15 support the hypothesis that the PC lipid-AZT conjugate compounds of the invention inhibit both infectious virus production and reverse transcriptase activity.

Example 2.

Sensitivity of AZT-Resistant Human Clinical Isolates of HIV-1 to PC Lipids Alone and Double Targeting PC lipid-AZT Conjugate Compounds

Sensitivity of AZT-resistant clinical isolates of HIV-1 to PC lipid alone (INK-17 and INK-18), non-PC lipid-AZT conjugate (INK-19) and a double-targeting PC lipid-AZT conjugate compound of the invention (INK-20) was evaluated in matched pairs of clinical isolates of HIV-1 obtained before ("pre-AZT") and after ("post-AZT") administration of AZT to HIV-1 infected humans. Isolates of HIV-1 obtained following administration of AZT included AZT-resistant virus particles. Evaluation of the clinical isolates to the compounds was performed using the plaque assay procedure described herein in Example 1. The matched pairs of clinical isolates were obtained through the AIDS Research and Reference Reagent Program, NIH, Bethesda, MD. The results of these assays are presented in Table 3. The data demonstrate that the double-targeting compound of the invention (INK-20) exhibited a much lower-fold increase in AZT-resistance among HIV-1 clinical isolates than did AZT alone (about 20-fold versus about 680- to 1,100-fold for AZT).

Table 3.

Compound	<u>EC₅₀ (μM)^a</u>		Fold-Increase	<u>EC₅₀ (μM)^a</u>		Fold-Increase
	<u>Pre-AZT</u> G-762	<u>Post-AZT</u> G-691		<u>Pre-AZT</u> H112-2	<u>Post-AZT</u> G-910	
INK-17	0.26	0.18	0	0.04	0.20	5.1
INK-18	>1.06	0.60	0	0.56	1.04	1.8
INK-19	0.12	1.62	13.5	0.03	>1.7	>55.6
INK-20	0.04	0.74	18.6	0.02	0.44	22.0
AZT	0.001	>1.29	>1,170	0.002	>1.36	>681.7

^a EC₅₀ values represent mean values calculated from 2 to 4 independent tests using duplicate wells for each of 4 serial concentrations of compound per test. ">" indicates that EC₅₀ was not achieved at the highest concentration tested.

Note--designations such as G-762 and H112-2 in the table designate patient codes for the source of the isolates.

Example 3

Anti-Herpes Simplex Virus Type 2 Activity of Double-Targeting
PC lipid-AZT Conjugate Compounds.

Proof-of-concept of the antiviral activity of the double-targeting PC lipid-AZT

5 conjugate compounds of the invention with respect to herpes simplex virus type 2 was evaluated and compared with that of PC lipid alone and with acyclovir (positive controls). Serial dilutions of PC lipid, PC lipid-AZT conjugate compound, or acyclovir were evaluated for inhibition of the formation of herpes simplex virus type 2 plaques in Vero cells and EC₅₀ values were calculated from the results obtained. Briefly, the tests for anti-herpes simplex virus 10 (HSV) activity and cytotoxicity of the compounds were performed by seeding 8 x 10⁴ monkey kidney cells (Vero) per ml of D-MEM with 10% fetal bovine serum (FBS) in each well of a 12-well dish. The cultures were incubated at 37°C to form a complete monolayer. To measure anti-HSV activity and effective concentration ₅₀ (EC₅₀) each cell monolayer was infected with HSV diluted in PBS-A containing about 100 plaque forming units (PFU) per 0.1 ml. After 15 virus attachment (1 hr., 37°C) the infected monolayers were overlaid with E-MEM containing 2% FBS and 0.5% methyl cellulose with or without added serial concentrations of test compound. After 2 days at 37°C to allow HSV induced plaque formation, the overlay medium was aspirated, the cell monolayers were fixed with 95% ethanol, stained with 0.1% crystal violet in 20% methanol and distilled water and the number of PFU in the compound treated 20 cultures was divided by the number of PFU in the untreated controls to determine the percent inhibition of PFU. The EC₅₀ values were calculated using a computer generated program.

To measure cytotoxicity of the compounds, the cell monolayers (described above) were treated with a serial concentration of test compound for 48 hours at 37°C and visually examined after crystal violet staining by light microscopy for changes in cell 25 morphology (cell rounding, shrinking, detachment) compared to an untreated control culture. The cytotoxicity (TC₅₀) represents the lowest serial concentration of test compound that caused a detectable change in cell morphology in at least 25% of the cells.

The results of these experiments are presented in Table 4. These results indicate that PC lipid-AZT conjugate compounds of the invention are metabolized intracellularly to 30 release two drugs which can target the virus life cycle. The PC lipid compound INK-24 and the AZT conjugate corresponding to INK-24 (i.e. INK-26) both exhibit selective activity against herpes simplex virus type 2, exhibiting EC₅₀ values of 13.8 and 12.0 micromolar, respectively.

By comparison, the range of EC₅₀ values for acyclovir were 12.5, 14.5 and 6.67 micromolar in replicate tests. Because herpes simplex virus is not expected to be inhibited by AZT, the observed inhibition of the virus by INK-26 is most likely due to intracellular metabolism of INK-26, leading to release of biologically active PC lipid. PC lipid should exhibit activity 5 against the herpes simplex virus in a manner and to a degree similar to the anti-herpes virus activity of INK-24. Cytotoxicity attributable to the double-targeting compound INK-26 appeared to be no worse than that of the positive control (acyclovir) and the PC lipid compound INK-24.

Table 4.

Compound	Cytotoxicity (TC ₅₀) (μ M)	Anti-Herpes Simplex Activity (EC ₅₀) ^a (μ M)
INK-17	toxic @ 4	>4
INK-18	ND	ND
INK-19	>20	>20
INK-20	toxic @ 20	>20
INK-21	toxic @ 20	>20
INK-22	toxic @ 20	>20
INK-23	>20	>20
INK-24	>20	13.9, 13.8
INK-25	toxic @ 20	>20
INK-26	>20	12.0
Acyclovir control	>20	14.5, 12.5, 6.67

10 ^a EC₅₀ values represent mean values obtained from 2 independent tests using duplicate wells for each of four serial concentrations of compound per test. ">" indicates that cytotoxicity or EC₅₀ was not achieved at the highest concentration tested.

Example 4

15 Data from testing in animals indicating that the phospholipid-AZT conjugate BM21-1290 (see Figure 1A) is orally bioavailable and preferentially taken up into lymphoid

tissues (lymphoma, spleen and thymus) is shown in Figure 5. Also, in rodents (mice) receiving oral administration of the conjugate, the compound has exhibited the ability to cross the blood-brain barrier and enter the brain. The data shown in Figure 6 indicates that the concentration of the conjugate compound BM21-1290 in the brain was found to be equivalent to the 5 concentration of BM21-1290 in the plasma. In rodent experiments there was little or no detectable free AZT in the plasma. These data suggest that as a phospholipid-AZT conjugate the AZT is protected from glucuronide formation in the liver. The half-life of the conjugate compound in rodents was 48 hours compared to 30 minutes to 1 hour for AZT alone.

10 In another set of experiments the metabolism of the compound BM21-1290 using human lymphocytes in tissue culture was assessed. Results of these experiments indicated that the conjugate is metabolized to form an alkyl-lipid plus a phosphorylated species of AZT (i.e., AZT-MP, AZT-DP, AZT-TP and AZT). The predominant AZT species was AZT-MP with lesser amounts of AZT-DP, AZT-TP and AZT. These data suggest that 15 phospholipid-AZT conjugates are metabolized intracellularly by a phospholipase C enzyme to yield a lipid and various species of AZT. AZT-MP can be activated to AZT-TP the inhibitor of HIV-1 induced reverse transcriptase.

20 Taken together, data from these experiments indicate that phospholipid-AZT conjugates are orally bioavailable, are preferentially taken up by lymphoid tissues, can cross the blood-brain barrier and are subsequently metabolized inside cells to yield an alkyl-lipid and AZT. Both the alkyl-lipid and AZT can function in double targeting the HIV-1 life cycle.

Example 5.

Synthesis of AZT-Malonic Acid

One gram of AZT was dissolved in 30 ml of anhydrous acetonitrile and added 25 dropwise to a solution of 632 mg of malonyl chloride in 20 ml of acetonitrile at 0°C. The reaction mixture was stirred for 2 hours at 0°C then at 8-10°C for 4.5 hours. Thin layer chromatography was used to indicate that the reaction was complete. Water (4 ml) was added. Solvents were removed in vacuo and the residue purified by silica gel chromatography eluting with CHCl₃:MeOH. Pure product was obtained in a 68% yield.

Example 6.

Synthesis of INK-20

The conditions for the first reaction of the synthesis method for INK-20 is described in Kucera et al., 1998, *Antiviral Chemistry & Chemotherapy*, 9: 157-165 at p.159 as the two-step synthesis of 3-dodecanamido-2-octyloxypropyl 2-bromethyl phosphate then 3-dodecanamido-2-octyloxypropyl phosphocholine (INK-3). However, the lipid used was 3-dodecylthio-2-(8'-benzyloxyoctyloxy)-1-propanol (see Figure 4) rather than 3-dodecanamido-2-octyloxy-1-propanol as described in the reference. The reactions were the same.

Hydrogenation with H₂-Pd/C was performed as follows. The phosphocholine [3-dodecylthio-2-(8'-benzyloxyoctyloxy)propyl phosphocholine] was dissolved in 60 ml absolute EtOH and

10 added to 109 mg of palladium black. This reaction mixture was shaken under 59 psi hydrogen gas for 24 hours. The catalyst was removed by filtration through Celite, solvent was removed in vacuo, and the residue was chromatographed on silica gel using CHCl₃:MeOH (100:0 to 2:1) as eluent to give 228 mg (43% yield) of [3-dodecylthio-2-(8'-hydroxyoctyloxy)propyl

15 phosphocholine]. AZT-MA (AZT-malonic acid) was then added by the following procedure: One gram of lipid, 192 mg of AZT-MA, 144 mg of dicyclohexylcarbodiimide (DCC), and 9 mg of dimethylaminopyridine (DMAP) were added to 10 ml of DMF. This reaction mixture was stirred at room temperature for 42 hours; copious solid appeared after 18 hours of stirring. The solid was filtered and the solvent removed in vacuo. The residue was chromatographed on silica gel using EtOAc:CHCl₃:MeOH (2:2:1) as eluent then CHCl₃:MeOH (4:1 to 2:1) to give 141 mg (36% yield) of INK-20 (R_f ~ 0.3 in CHCl₃:MeOH:NH₄OH 20 75:25:5).

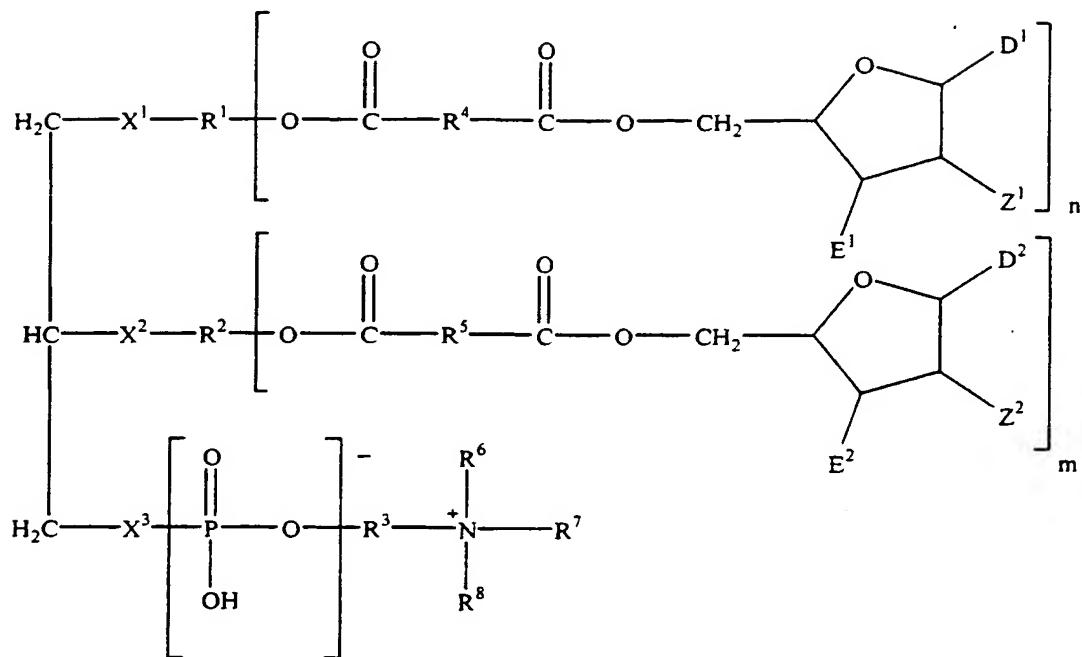
The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

25 While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention can be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A compound having the structure of Formula I:



wherein,

n and m are each independently 0 or 1, but n and m are not both 0;

R¹ is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl if n is 0 and

10 (C₁-C₁₆) alkylene, branched alkyl, alkenyl or alkynyl if n is 1;

R² is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl if m is 0 and (C₁-C₁₆) alkylene, branched alkyl, alkenyl or alkynyl if m is 1;

R³, R⁴ and R⁵ are each independently (C₁-C₈) alkylene;

R⁶, R⁷ and R⁸ are each independently (C₁-C₈) alkyl;

15 X¹ and X² are each independently S, O, NHC=O, OC=O or NH;

X³ is O or S;

E¹ is H, S, halo or N₃;

Z¹ is H, S, or halo; or E¹ and Z¹ together are a covalent bond;

E² is H, S, halo, or N₃;

20 Z² is H, S, or halo; or E² and Z² together are a covalent bond;

D¹ and D² are each independently selected from the group consisting of purine, pyrimidine, adenine, thymine, cytosine, guanine, hypoxanthine, inosine, uracil and ring modifications thereof, including O, N, and S substitutions, and

5 wherein, each alkyl, alkylene, branched alkyl, alkenyl, alkynyl, adenine, thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, D¹, and D² can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

10

2. The compound of claim 1, wherein said compound is present in an amount effective to inhibit virus replication in a mammal.

15

3. The compound of claim 1, wherein R¹ is (C₆-C₁₆) alkyl if n is 0 or -CH=CH- if n is 1.

4. The compound of claim 1, wherein R² is (C₆-C₁₆) alkyl if m is 0 or -CH=CH- if m is 1.

20

5. The compound of claim 1, wherein R³ is -CH₂CH₂-.

6. The compound of claim 1, wherein R⁴ is -CH₂-.

7. The compound of claim 1, wherein R⁵ is -CH₂-.

25

8. The compound of claim 1, wherein R⁶, R⁷ and R⁸ are each -CH₃.

9. The compound of claim 1, wherein X¹ is S, NHC=O, -NH- or O.

30

10. The compound of claim 1, wherein X² is S, NHC=O or O.

11. The compound of claim 1, wherein X³ is O or S.

12. The compound of claim 1, wherein E¹ is N₃, S or H.

13. The compound of claim 1, wherein Z¹ is H or S.

5

14. The compound of claim 1, wherein E² is N₃, S or H.

15. The compound of claim 1, wherein Z² is H or S.

10

16. The compound of claim 1, wherein n is 0 and m is 1.

17. The compound of claim 1, wherein n is 1 and m is 0.

18. The compound of claim 1, wherein D¹ is selected from the group consisting
15 of cytosine, guanine, inosine and thymine.

19. The compound of claim 1, wherein D² is selected from the group consisting
of cytosine, guanine, inosine and thymine.

20

20. A pharmaceutically acceptable salt of the compound of claim 1.

21. The pharmaceutically acceptable salt of claim 20, wherein said compound is
present in an amount effective to inhibit virus replication in a mammal.

25

22. The compound of claim 1,

wherein,

R¹ is (C₆-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl;

R² is (C₄-C₁₂) alkylene;

R³ is -CH₂CH₂-;

R⁵ is -CH₂-;

R⁶, R⁷ and R⁸ are each CH₃;

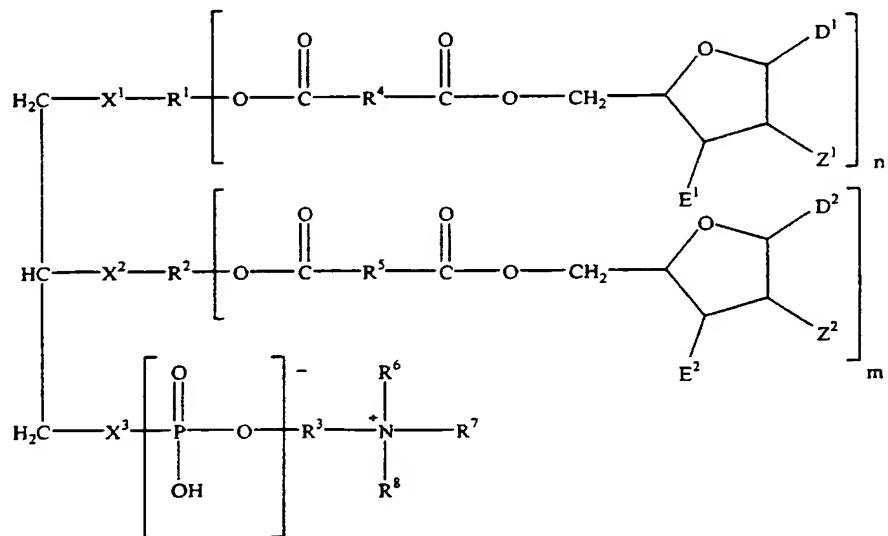
X¹ and X² are each independently S, O or NHC=O;

30

E^2 is H or N_3 ;

D² is selected from the group consisting of thymine, cytosine, guanine and inosine, and wherein each alkyl, branched alkyl, alkylene, alkenyl, alkynyl, thymine, cytosine, guanine, and inosine of R¹, R², R³, R⁵, R⁶, R⁷, R⁸, and D² can optionally be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b), wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

23. A method of treating a virus infection in a mammal, the method comprising
10 administering to said mammal, in an amount effective to treat said infection, a compound
having the structure of Formula I:



wherein,

n and m are each independently 0 or 1, but n and m are not both 0;

15 R¹ is (C₁-C₁₆) alkyl, branched alkyl, alkenyl or alkynyl if n is 0 and (C₁-C₁₆) alkylene, branched alkyl, alkenyl or alkynyl if n is 1;

R^2 is (C_1 - C_{16}) alkyl, branched alkyl, alkenyl or alkynyl if m is 0 and (C_1 - C_{16}) alkylene, branched alkyl, alkenyl or alkynyl if m is 1;

R^3 , R^4 and R^5 are each independently (C_1 - C_8) alkylene;

20 R⁶, R⁷ and R⁸ are each independently (C₁-C₈) alkyl;

X^1 and X^2 are each independently S, O, NHC=O, OC=O or NH;

X³ is O or S;

E¹ is H, S, halo or N₃;

Z¹ is H, S, or halo; or E¹ and Z¹ together are a covalent bond;

E² is H, S, halo, or N₃;

Z² is H, S, or halo; or E² and Z² together are a covalent bond;

5 D¹ and D² are each independently selected from the group consisting of purine, pyrimidine, adenine, thymine, cytosine, guanine, hypoxanthine, inosine, uracil and ring modifications thereof, including O, N, and S substitutions, and

10 wherein, each alkyl, alkylene, branched alkyl, alkenyl, alkynyl, adenine, thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, D¹, and D² can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C₁-C₈) alkyl, (C₁-C₈) alkoxy, aryl, and N(R^a)(R^b) wherein R^a and R^b are each independently selected from the group consisting of H and (C₁-C₈) alkyl.

15 24. The method of claim 23, wherein R¹ is (C₈-C₁₂) alkyl if n is 0 and -CH₂CH₂- if n is 1.

20 25. The method of claim 23, wherein R² is (C₈-C₁₂) alkyl if m is 0 and -CH₂CH₂- if m is 1.

26. The method of claim 23, wherein R³ is -CH₂CH₂-.

27. The method of claim 23, wherein R⁴ is -CH₂-.

25 28. The method of claim 23, wherein R⁵ is -CH₂-.

29. The method of claim 23, wherein R⁶, R⁷ and R⁸ are each -CH₃.

30 30. The method of claim 23, wherein X¹ is S or O.

30 31. The method of claim 23, wherein X² is S or O.

32. The method of claim 23, wherein X³ is O.

33. The method of claim 23, wherein E¹ is N₃ or H.

5 34. The method of claim 23, wherein Z¹ is H.

35. The method of claim 23, wherein E² is N₃ or H.

36. The method of claim 23, wherein Z² is H.

10 37. The method of claim 23, wherein n is 0 and m is 1.

38. The method of claim 23, wherein n is 1 and m is 0.

15 39. The method of claim 23, wherein D¹ is selected from the group consisting of cytosine, guanine, inosine, and thymine.

40. The method of claim 23, wherein D² is selected from the group consisting of cytosine, guanine, inosine, and thymine.

20 41. The method of claim 23, wherein said virus infection is an infection by a virus selected from the group consisting of HIV, hepatitis virus, and a herpes virus.

25 42. The method of claim 41, wherein said HIV is selected from the group consisting of HIV-1 and HIV-2.

43. The method of claim 41, wherein said hepatitis virus is selected from the group consisting of hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E viruses.

30 44. The method of claim 41, wherein said herpes virus is selected from the group consisting of herpes simplex virus type 1, herpes simplex virus type 2, varicella-zoster

virus, cytomegalovirus, Epstein Barr virus, human herpes virus type 6, human herpes virus type 7, and human herpes virus type 8.

45. The method of claim 23,

5 wherein

R^1 is (C_6-C_{16}) alkyl, branched alkyl, alkenyl or alkynyl;

R^2 is (C_4-C_{12}) alkylene;

R^3 is $-CH_2CH_2-$;

R^5 is $-CH_2-$;

10 R^6 , R^7 and R^8 are each CH_3 ;

X^1 and X^2 are each independently S, O or $NHC=O$;

E^2 is H or N_3 ;

15 D^2 is selected from the group consisting of thymine, cytosine, guanine and inosine, and wherein each alkyl, branched alkyl, alkylene, alkenyl, alkynyl, thymine, cytosine, guanine, and inosine of R^1 , R^2 , R^3 , R^5 , R^6 , R^7 , R^8 , and D^2 can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C_1-C_8) alkyl, (C_1-C_8) alkoxy, aryl, and $N(R^a)(R^b)$, wherein R^a and R^b are each independently selected from the group consisting of H and (C_1-C_8) alkyl.

20 46. The method of claim 23, wherein a pharmaceutically acceptable salt of said compound is administered to said mammal.

47. The method of claim 23, wherein said mammal is a human.

25 48. A method of inhibiting virus replication in a cell, the method comprising administering to said cell a compound of claim 1 in an amount effective to inhibit virus replication in said cell.

30 49. A pharmaceutical composition comprising a compound of claim 1 in combination with a pharmaceutically acceptable carrier.

50. A pharmaceutical composition comprising a compound of claim 22 in combination with a pharmaceutically acceptable carrier.

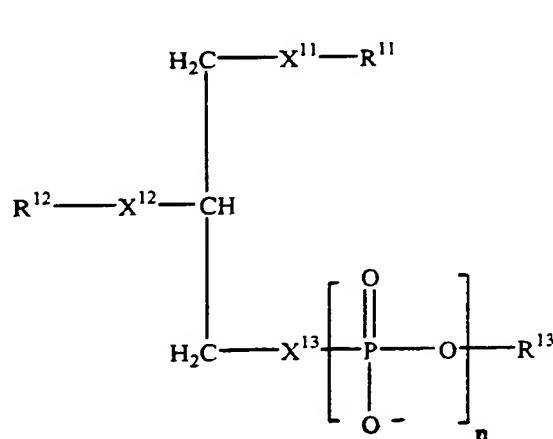
51. The pharmaceutical composition of claim 49, wherein said compound is present in an amount effective to inhibit virus replication in a mammal.

52. The pharmaceutical composition of claim 50, wherein said compound is present in an amount effective to inhibit virus replication in a mammal.

10 53. A kit for treatment of a viral infection in a mammal, said kit comprising
a) a composition selected from the group consisting of a compound of claim 1, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising a compound of claim 1, and
b) an instructional material.

15 54. A kit for inhibition of virus replication in a cell, said kit comprising
a) a composition selected from the group consisting of a compound of claim 1, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising a compound of claim 1, and
b) an instructional material.

20 55. A compound having the structure of Formula III:



25

(III)

wherein,

R^{11} is (C_1-C_{16}) alkyl, branched alkyl, alkenyl or alkynyl;

R^{12} is (C_1-C_{16}) alkyl, branched alkyl, alkenyl or alkynyl;

X^{11} is O, S, or $NHC=O$;

5 X^{12} is O, S, or $NHC=O$;

X^{13} is O or S;

n is 0, 1 or 2, and

R^{13} is a therapeutic agent,

wherein, each alkyl, branched alkyl, alkenyl, alkynyl, adenine, thymine,

10 cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^{11} , R^{12} , and R^{13} can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C_1-C_8) alkyl, (C_1-C_8) alkoxy, aryl, and $N(R^a)(R^b)$ wherein R^a and R^b are each independently selected from the group consisting of H and (C_1-C_8) alkyl, and

15 wherein, if n is 1 or 2, the compound is a phospholipase C substrate and is not a phospholipase A substrate, and

further wherein, if n is 1 or 2, the compound is converted to an alkyl lipid and a moiety selected from the group consisting of a nucleoside monophosphate and a nucleoside analogue monophosphate intracellularly in a mammal, and is not converted to an alkyl lipid and

20 a moiety selected from the group consisting of a nucleoside monophosphate and a nucleoside analogue monophosphate extracellularly in a mammal.

56. The compound of claim 55,

wherein,

25 R^{11} is a C_{12} alkyl, branched alkyl, alkenyl or alkynyl;

R^{12} is C_8H_{16} alkyl or branched alkyl;

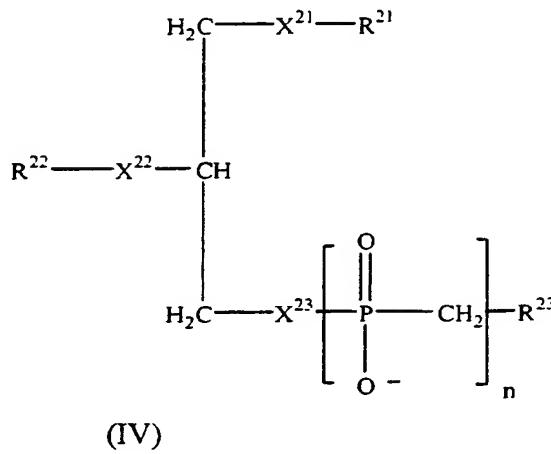
$n = 1$,

and R^{13} is an anticancer agent selected from the group consisting of gemcitabine,

ara-C, 5-azacytidine, cladribine, flucytosine, fluorodeoxyuridine, cytosine arabinoside and 6-

30 mercaptopurine, wherein the phosphorus atom of the phosphate moiety is covalently linked in a phosphate ester linkage to the oxygen atom of the 5' hydroxyl group of a sugar moiety of R^{13} .

57. A compound having the structure of Formula IV:



5 wherein,

R^{21} is (C_6 to C_{16}) alkyl, branched alkyl, alkenyl, or alkynyl;

R^{22} is (C_1 to C_{12}) alkyl, branched alkyl, alkenyl, or alkynyl;

X^{21} is O, S, or NHC=O ;

X^{22} is O, S, or NHC=O ;

10 X^{23} is O or S;

n is 1 or 2;

R^{23} is a therapeutic agent, and

wherein, each alkyl, branched alkyl, alkenyl, alkynyl, adenine, thymine,

cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^{21} , R^{22} , and R^{23} can,

15 optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C_1 - C_8) alkyl, (C_1 - C_8) alkoxy, aryl, and $\text{N}(\text{R}^a)(\text{R}^b)$ wherein R^a and R^b are each independently selected from the group consisting of H and (C_1 - C_8) alkyl.

20 58. The compound of claim 57,

wherein,

R^{21} is C_{12} alkyl;

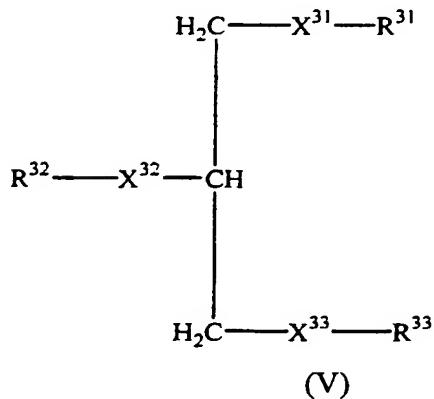
R^{22} is C_{10} alkyl;

n = 1, and

R^{23} is an anticancer agent selected from the group consisting of gemcitabine, ara-C, 5-azacytidine, cladribine, flucytosine, fluorodeoxyuridine, cytosine arabinoside and 6-mercaptopurine, wherein the methylene group of the phosphonate moiety is covalently linked to the oxygen atom of the 5' hydroxyl group of a sugar moiety of R^{23} .

5

59. A compound having the structure of Formula V:



10 wherein,

R^{31} is (C_1 to C_{16}) alkyl, branched alkyl, alkenyl, or alkynyl;

R^{32} is (C_1 to C_{16}) alkyl, branched alkyl, alkenyl, or alkynyl;

X^{31} is O, S, or NHC=O ;

X^{32} is O, S, or NHC=O ;

15 X^{33} is $-\text{OH}$, $-\text{SH}$, or amino;

R^{33} is a therapeutic agent, and

wherein, each alkyl, branched alkyl, alkenyl, alkynyl, adenine, thymine, cytosine, guanine, pyrimidine, purine, hypoxanthine, inosine and uracil of R^{31} , R^{32} , and R^{33} can, optionally, be substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of halo, nitro, trifluoromethyl, (C_1 - C_8) alkyl, (C_1 - C_8) alkoxy, aryl, and $N(R^a)(R^b)$ wherein R^a and R^b are each independently selected from the group consisting of H and (C_1 - C_8) alkyl.

60. The compound of claim 59,

25 wherein,

R^{31} is (C_6 - C_{16}) alkyl, branched alkyl, alkenyl or alkynyl;

R^{32} is $(C_1 - C_8)$ alkyl, branched alkyl, alkenyl or alkynyl, and

R^{33} is an anticancer agent selected from the group consisting of mitoxanthrone, methotrexate and CPT-11, and is covalently linked via an ester, amido or carbamate linkage to the $-SH$, OH or amino group of X^{33} .

5

61. The compound of claim 55, wherein said compound is suspended in a pharmaceutically acceptable carrier and is present in an amount effective to combat a cancer in a mammal.

10

62. The compound of claim 61, wherein said cancer is a cancer selected from the group consisting of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma and a solid tumor.

15

63. The compound of claim 55, wherein said compound is present in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

64. The compound of claim 63, wherein said therapeutic agent is an anticancer agent.

20

65. The compound of claim 63, wherein the cell is in a mammal.

66. The compound of claim 65, wherein the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

25

67. The compound of claim 66, wherein the CNS cell is an astrocyte or a glial cell.

68. A pharmaceutically acceptable salt of the compound of claim 55.

30

69. The pharmaceutically acceptable salt of claim 68, wherein the compound is present in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

70. The pharmaceutically acceptable salt of claim 69, wherein the cell is in a mammal.

5 71. The pharmaceutically acceptable salt of claim 70, wherein the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

72. The pharmaceutically acceptable salt of claim 68, wherein said compound is present in an amount effective to combat a cancer in a mammal.

10 73. A pharmaceutically acceptable salt of the compound of claim 56.

74. The pharmaceutically acceptable salt of claim 73, wherein said compound is present in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

15 75. The pharmaceutically acceptable salt of claim 74, wherein said therapeutic agent is an anticancer agent.

76. The pharmaceutically acceptable salt of claim 74, wherein said cell is in a mammal.

20 77. The pharmaceutically acceptable salt of claim 74, wherein said cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

25 78. The pharmaceutically acceptable salt of claim 68, wherein said compound is present in an amount effective to combat a cancer in a mammal.

79. A drug delivery agent comprising a pharmaceutical composition, said composition comprising a compound of claim 55 or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

30 80. The drug delivery agent of claim 79, wherein said therapeutic agent is an anticancer agent.

81. The drug delivery agent of claim 79, wherein said cell is in a mammal.

5 82. The drug delivery agent of claim 79, wherein said cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

10 83. A drug delivery agent comprising a pharmaceutical composition, said composition comprising a compound of claim 55 or a pharmaceutically acceptable salt thereof, in an amount effective to combat a cancer in a mammal.

15 84. The drug delivery agent of claim 83, wherein said cancer is a cancer selected from the group consisting of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma and a solid tumor.

20 85. A drug delivery agent comprising a pharmaceutical composition, the composition comprising a compound of claim 56 or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of a therapeutic agent to a mammalian cell.

25 86. The drug delivery agent of claim 85, wherein the cell is in a mammal.

30 87. The drug delivery agent of claim 85, wherein said cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

88. A drug delivery agent comprising a pharmaceutical composition, said composition comprising a compound of claim 56 or a pharmaceutically acceptable salt thereof, in an amount effective to combat a cancer in a mammal.

89. The drug delivery agent of claim 88, wherein said cancer is a cancer selected from the group consisting of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma and a solid tumor.

90. A method of facilitating delivery of a therapeutic agent to a mammalian cell, said method comprising administering to said cell a pharmaceutical composition comprising a compound of claim 55 or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of said therapeutic agent to said cell.

5

91. The method of claim 90, wherein said therapeutic agent is an anticancer agent.

92. The method of claim 90, wherein said cell is in a mammal.

10

93. The method of claim 90, wherein the cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

94. A method of facilitating delivery of a therapeutic agent to a cell, said method comprising administering to said cell a pharmaceutical composition comprising a compound of claim 56 or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of said therapeutic agent to said cell.

95. The method of claim 94, wherein said cell is in a mammal.

20

96. The method of claim 94, wherein said cell is a cell selected from the group consisting of a CNS cell and a lymphoid cell.

97. A method of combating a cancer in a mammal comprising administering to said mammal a pharmaceutical composition comprising a compound of claim 55 or a pharmaceutically acceptable salt thereof, in an amount effective to combat a cancer in the mammal.

98. The method of claim 97, wherein said cancer is a cancer selected from the group consisting of a carcinoma, a sarcoma, a neuroblastoma, a leukemia, a lymphoma and a solid tumor.

99. A method of treating a disease in a mammal, said method comprising administering to said mammal a pharmaceutical composition comprising a compound of claim 55, or a pharmaceutically acceptable salt thereof, in an amount effective to facilitate delivery of a therapeutic agent to a cell in said mammal, thereby treating said disease.

5

100. The method of claim 99, wherein said disease is a disease selected from the group consisting of a brain disease, a CNS disease, a lymphatic system disease, a reproductive system disease, a cardiovascular disease, a kidney disease and a liver disease.

10

101. A kit for combating a cancer in a mammal, said kit comprising

a) a composition selected from the group consisting of a compound of claim 55, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising a compound of claim 55, and

b) an instructional material.

15

102. A kit for facilitating delivery of a therapeutic agent to a mammalian cell, said kit comprising

a) a composition selected from the group consisting of a compound of claim 55, a pharmaceutically acceptable salt thereof, and a pharmaceutical composition comprising a compound of claim 55, and

b) an instructional material.

103. The kit of claim 102, wherein said therapeutic agent is an anticancer agent.

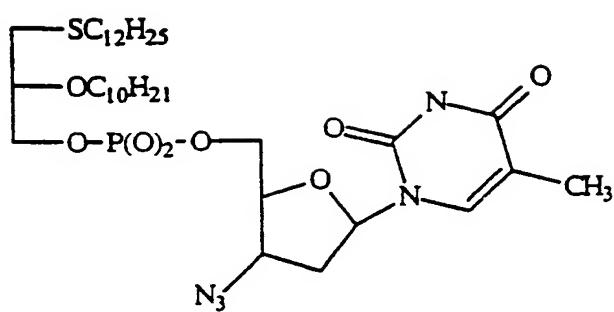


FIGURE 1A

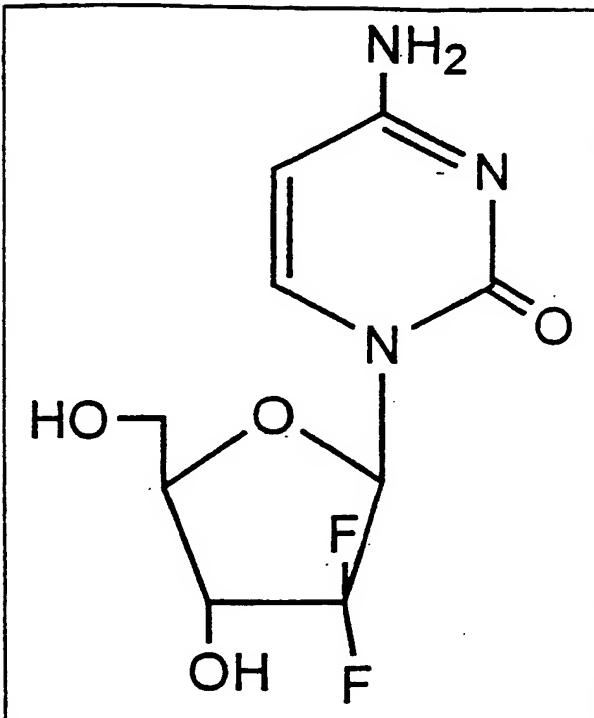


FIGURE 1B

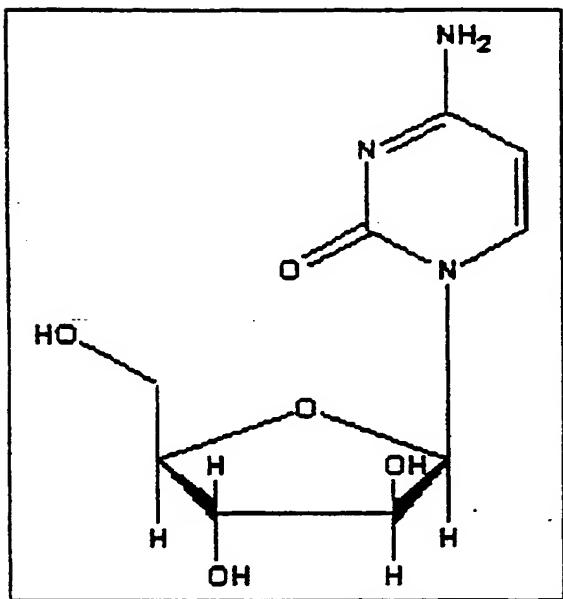


FIGURE 1C

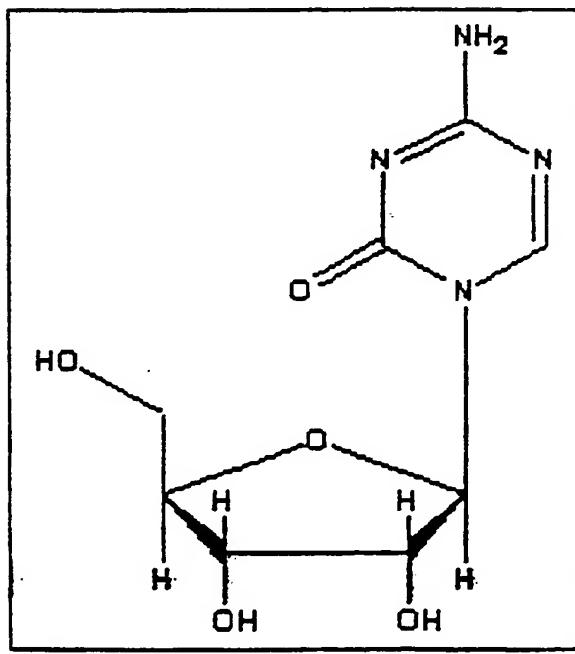


FIGURE 1D

Synthesis of Lipid Backbone

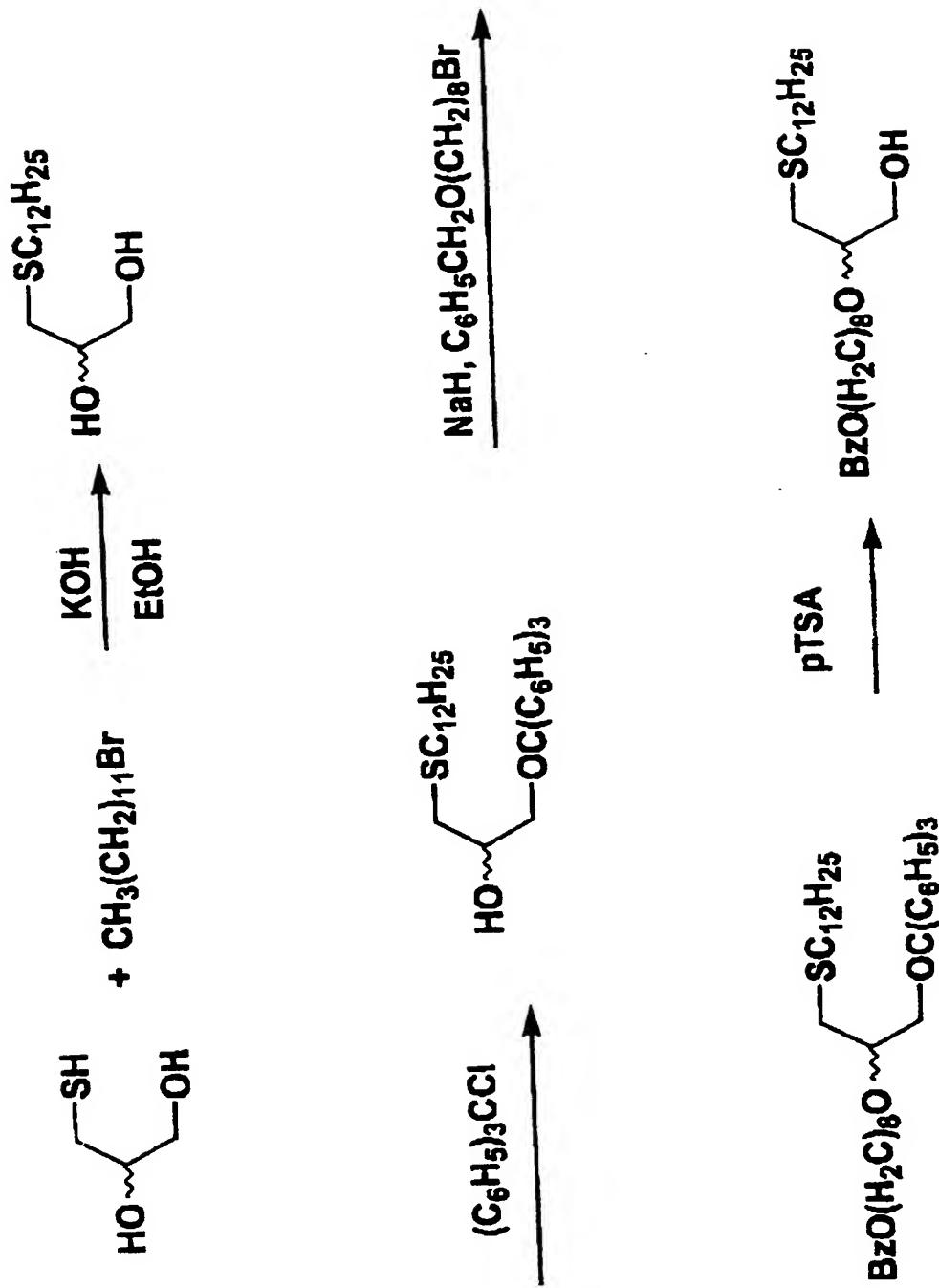
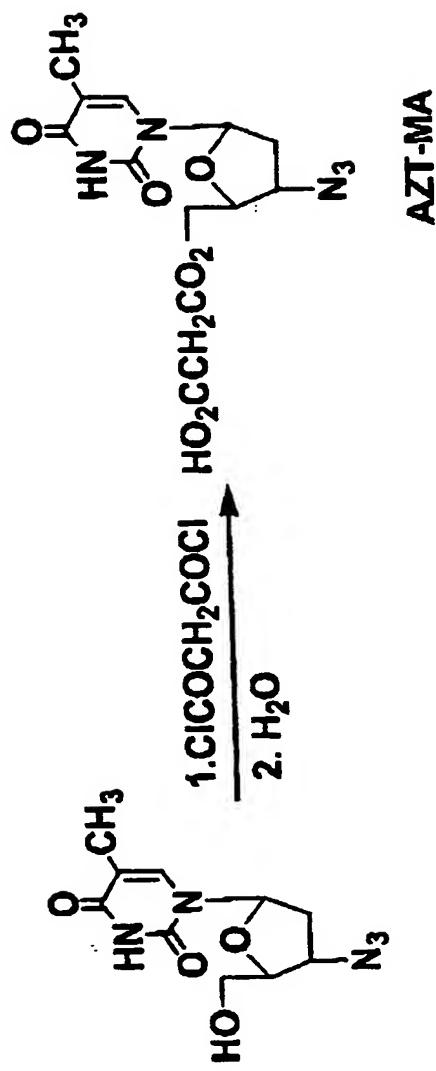


FIGURE 2

Synthesis of AZT-Malonic Acid (AZT-MA)

Substituted malonic acid chlorides (ClCOCHRCOCl) could also be used in the above reaction;
 $R = \text{CH}_3, \text{CH}_2\text{CH}_3, \text{C}_6\text{H}_5$

FIGURE 3

Synthesis of AZT-Phosphocholine Conjugate

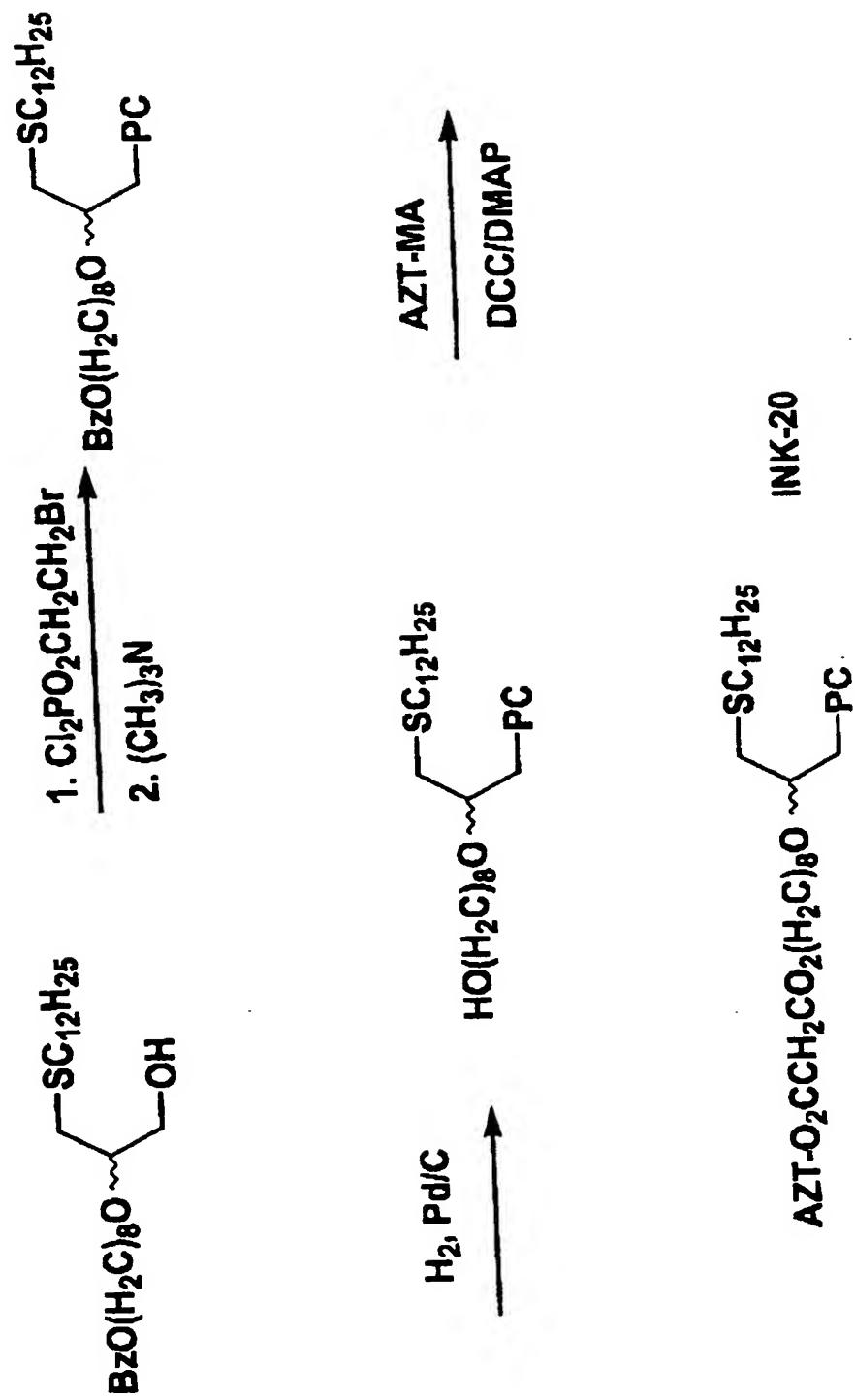


FIGURE 4

[¹⁴C]-BM 21.1290 concentrations in plasma and lymphoid tissues of female C57Bl/6 mice

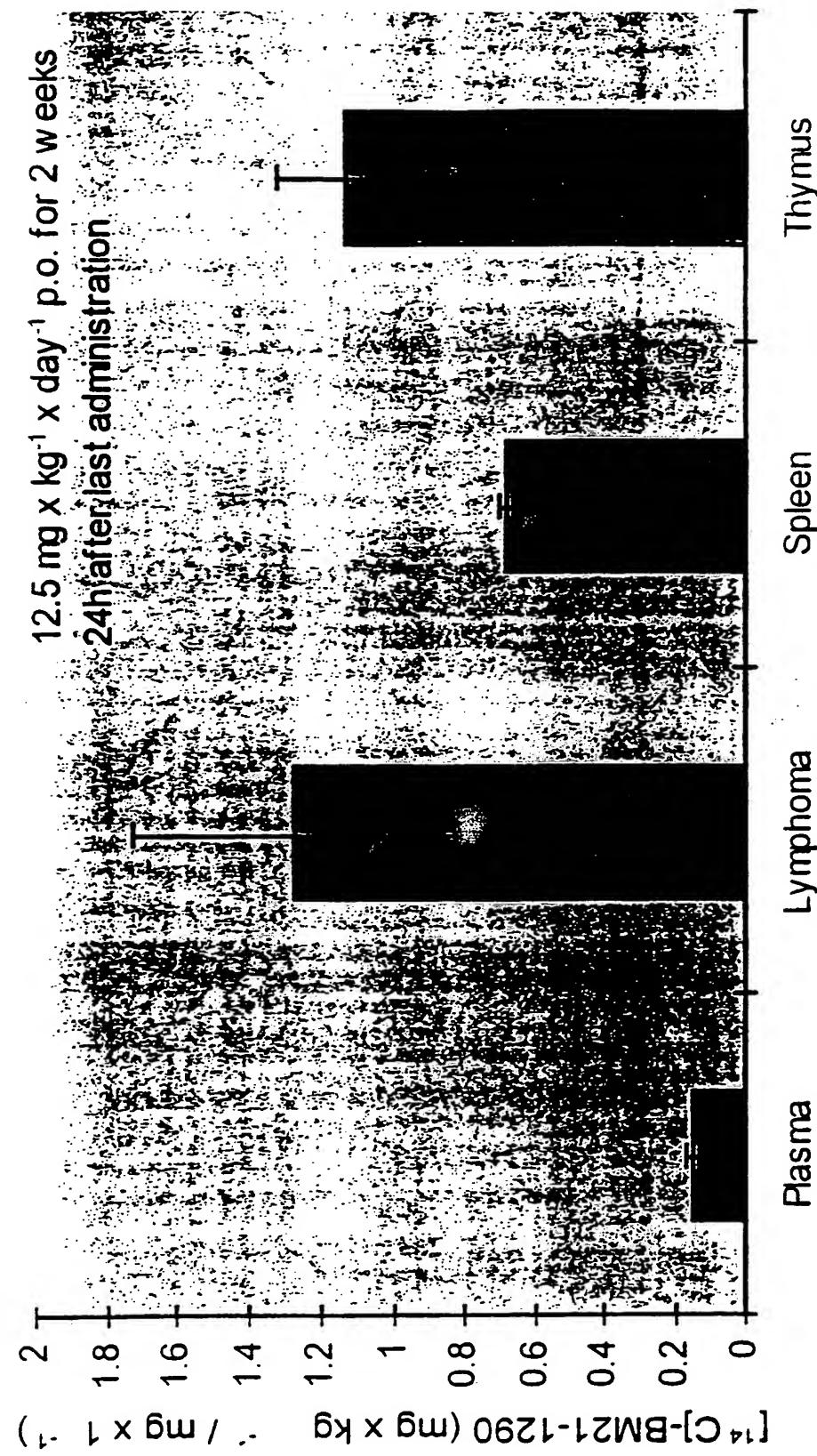


FIGURE 5

Plasma and CNS concentrations of [^{14}C]-BM 21.1290 in
female C57Bl/6 mice

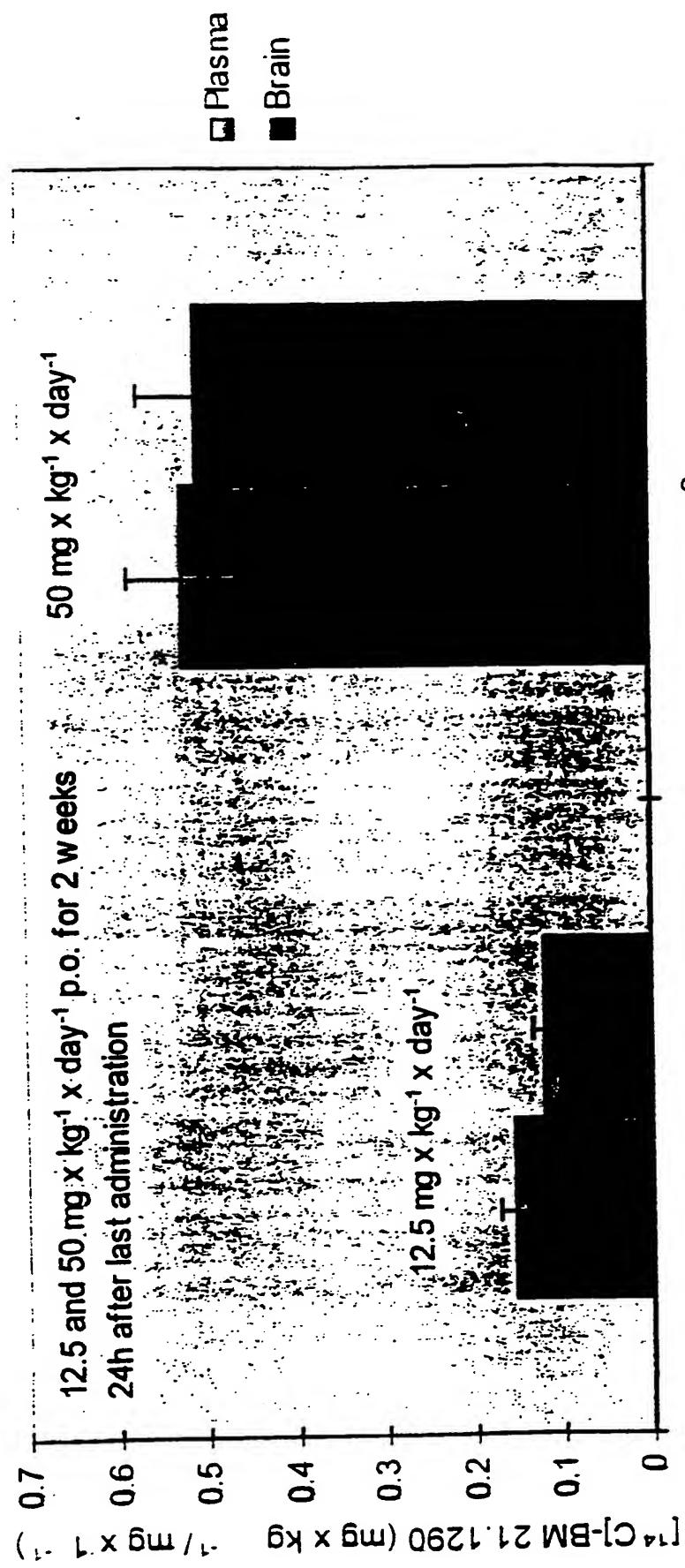


FIGURE 6

lipid & ara-C coupled through phosphate ester

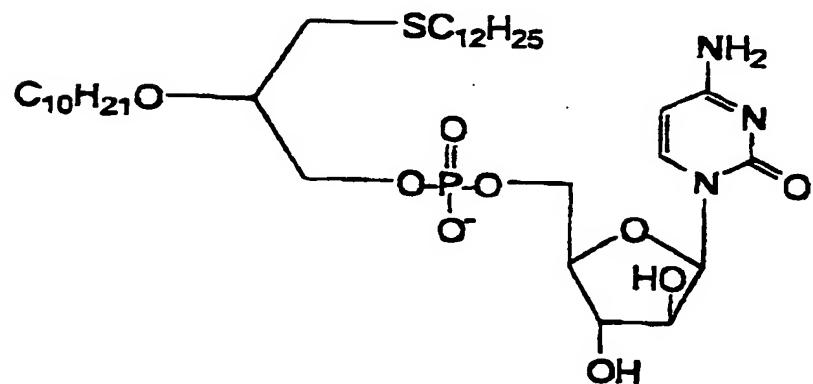


FIGURE 7A

lipid & gemcitabine coupled through phosphate ester

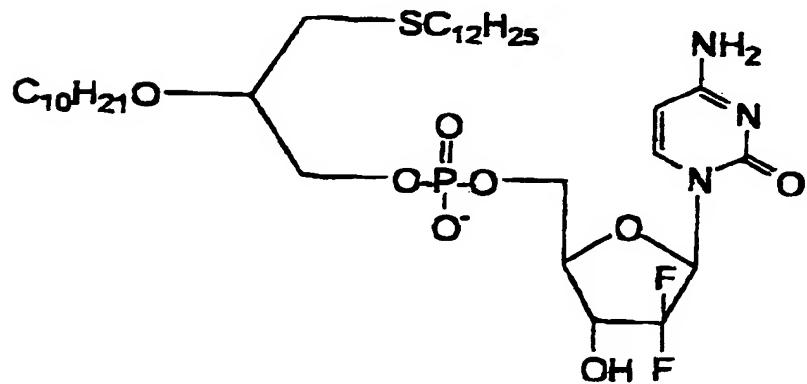


FIGURE 7B

lipid & ara-C coupled through phosphonate ester

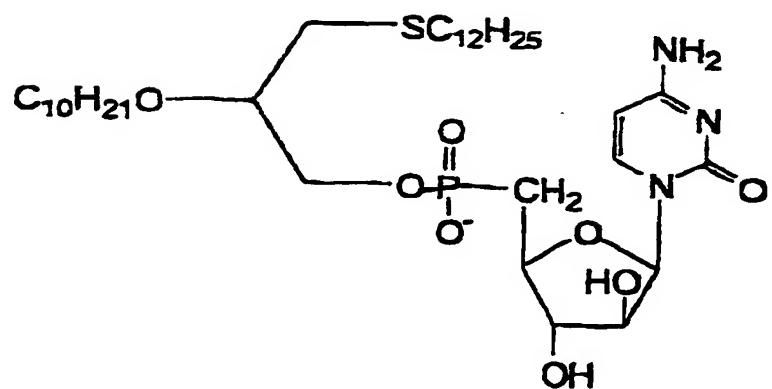


FIGURE 8A

lipid & gemcitabine coupled through phosphonate ester

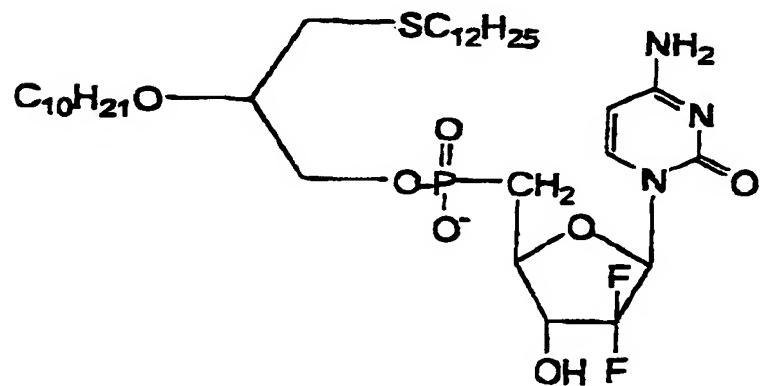


FIGURE 8B

lipid coupled to methotrexate through an ester

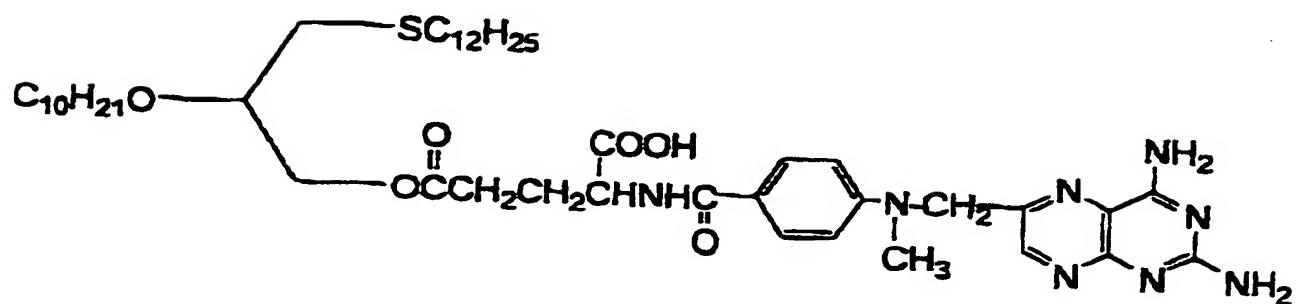


FIGURE 9

THIS PAGE BLANK (USPTO)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/34614 A3(51) International Patent Classification⁷: C07H 19/06.
19/16, C07F 9/10, A61K 31/7052, 31/685, A61P 31/12,
35/00Susan, L. [US/US]: 1225 Martha's Chapel Road, Rt. 3,
Box 184P, Apex, NC 27502 (US).

(21) International Application Number: PCT/US00/41352

(74) Agents: DOYLE, Kathryn et al.: Morgan, Lewis & Bockius LLP, 1701 Market Street, Philadelphia, PA 19103 (US).

(22) International Filing Date: 20 October 2000 (20.10.2000)

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/162,290 28 October 1999 (28.10.1999) US(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicants (for all designated States except US): WAKE FOREST UNIVERSITY [US/US]: Medical Center Boulevard, Winston-Salem, NC 27157-1023 (US). THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL [US/US]: Office of Technology Development, 308 Bynum Hall, Campus Box 4105, Chapel Hill, NC 27599-4105 (US).

Published:

— with international search report

(72) Inventors; and

(75) Inventors/Applicants (for US only): KUCERA, Louis, S. [US/US]: 4860 Ellen Avenue, Pfafftown, NC 27040 (US). FLEMING, Ronald, A. [US/US]: 1005 Greenly Drive, Pfafftown, NC 27040 (US). ISHAQ, Khalid, S. [US/US]: 105 Hunter Hill Place, Chapel Hill, NC 27514 (US). KUCERA, Gregory, L. [US/US]: 705 Manly Street, Winston-Salem, NC 27101 (US). MORRIS-NATSCHKE,

(88) Date of publication of the international search report:
27 December 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPOSITIONS AND METHODS FOR DOUBLE-TARGETING VIRUS INFECTIONS AND TARGETING CANCER CELLS

WO 01/34614 A3

(57) Abstract: The invention includes compositions and methods useful for treatment of a virus infection in a mammal by double-targeting the virus (i.e. targeting the virus at more than one stage of the virus life cycle) and thereby inhibiting virus replication. The compositions of the invention include compounds which comprise a phosphocholine moiety covalently conjugated with one or more antiviral agents (e.g. nucleoside analogue, protease inhibitor, etc.) to a lipid backbone. The invention also includes pharmaceutical compositions and kits for use in treatment of a virus infection in mammals. The methods of the invention comprise administering a compound of the invention, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the invention, in an amount effective to treat the infection, to a mammal infected with a virus. Additionally, the invention includes compositions and methods useful for combating a cancer in a mammal and for facilitating delivery of a therapeutic agent to a mammalian cell. The compositions of the invention include compounds which comprise an alkyl lipid or phospholipid moiety covalently conjugated with an anticancer agent (e.g. a nucleoside analogue). The invention also includes pharmaceutical compositions and kits for combating a cancer and for facilitating delivery of a therapeutic agent to a mammalian cell. The methods of the invention comprise administering a compound of the invention, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the invention, in an amount effective to combat a cancer or to facilitate delivery of a therapeutic agent to a mammalian cell.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 00/41352

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C07H19/06	C07H19/16	C07F9/10	A61K31/7052	A61K31/685
A61P31/12	A61P35/00				

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H C07F A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 88 03146 A (FUJI CHEM IND CO LTD) 5 May 1988 (1988-05-05) abstract ---	1,2,20, 23
A	WO 91 19726 A (UNIV WAKE FOREST ;UNIV NORTH CAROLINA (US)) 26 December 1991 (1991-12-26) the whole document ---	1,23
A	WO 96 06620 A (MORRIS NATSCHKE SUSAN L ;UNIV NORTH CAROLINA (US); ISHAQ KHALID S) 7 March 1996 (1996-03-07) claims 59-74 ---	1,23



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

17 May 2001

Date of mailing of the international search report

14 08. 2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel: (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

de Nooy, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/41352

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PIANTADOSI C ET AL: "SYNTHESIS AND EVALUATION OF NOVEL ETHER LIPID NUCLEOSIDE CONJUGATES FOR ANTI-HIV-1 ACTIVITY" JOURNAL OF MEDICINAL CHEMISTRY, US, AMERICAN CHEMICAL SOCIETY, WASHINGTON, vol. 34, no. 4, 1991, pages 1408-1414, XP002000878 ISSN: 0022-2623 cited in the application the whole document --- 	1,23
A	HONG C I ET AL: "FORMULATION, STABILITY, AND ANTITUMOR ACTIVITY OF 1-BETA-D-ARABINOFURANOSYLCYTOSINE CONJUGATE OF THIOETHER PHOSPHOLIPID" CANCER RESEARCH, US, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 50, no. 14, 15 July 1990 (1990-07-15), pages 4401-4406, XP000571731 ISSN: 0008-5472 cited in the application abstract figure 1 ----- 	1,23

INTERNATIONAL SEARCH REPORT

Int. application No.
PCT/US 00/41352

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-54

Remark on Protest

The additional search fees were accompanied by the applicant's protest

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-54

Compounds of Formula I of claim 1, methods, compositions and kits related thereto.

2. Claims: 55-56, 61-103

Compounds of Formula III of claim 55, salts, methods, compositions and kits related thereto.

3. Claims: 57-58

Compounds of Formula IV of claim 57.

4. Claims: 59-60

Compounds of Formula V of claim 59.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/41352

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 8803146 A	05-05-1988	JP	61246196 A	01-11-1986
WO 9119726 A	26-12-1991	AT	137242 T	15-05-1996
		AU	660417 B	29-06-1995
		AU	8059791 A	07-01-1992
		CA	2085354 A	16-12-1991
		DE	69119074 D	30-05-1996
		DE	69119074 T	12-12-1996
		EP	0533825 A	31-03-1993
WO 9606620 A	07-03-1996	AU	3216695 A	22-03-1996
		CA	2197319 A	07-03-1996
		EP	0781138 A	02-07-1997
		JP	10506619 T	30-06-1998
		US	5962437 A	05-10-1999

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)